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Agriculture Development Fund

FINAL REPORT

ENHANCING STRESS TOLERANCE OF HIGH-VALUE CROPS USING ABA ANALOGS

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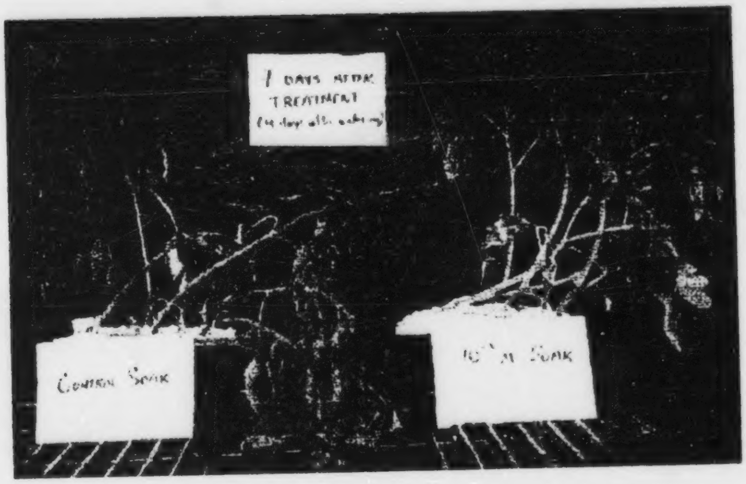
Final Report

October 1, 2000

Evaluation of ABA analogues for Enhancement of Stress Tolerance in High-Value Horticultural Crops

ADF PROJECT 97000289


7 DAYS AFTER TREATMENT
(14 days after soaking)
Control Soak
10 M Soak

Prepared by: Doug Waterer
Vegetable Crops Specialist
University of Saskatchewan

ABA Analogues and Stress Tolerance of Horticultural Crops - ADF Project 97000289

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ABA Analogues and Stress Tolerance of Horticultural Crops
ADF Project No. 97000289

Abstract

Drought shock and cold stress are common causes of loss during establishment of horticultural crops such as vegetables, ornamentals and flowering annuals. Application of **abscisic acid (ABA)** to young seedlings or transplants can increase their tolerance to drought stress and cold. Unfortunately, the beneficial effects of ABA applied in this way are short lived as the chemical is rapidly metabolized. PBI/NRC has generated ABA analogs which are more physiologically active and longer-lasting than standard ABA. This research program tested several practical applications of the ABA analogs in high-value Horticultural crops. Specifically, this project ......

a) evaluated the efficacy of the most promising ABA analogs in alleviating cold and drought stress in transplants of high-value Horticultural crops. The ABA analogs were more effective for protection from drought stress than from cold. A series of studies determined optimum dosages and time of application, effective duration of protection and potential to enhance yield and profitability while reducing production risks

b) showed that when applied at appropriate dosages, the ABA analogs enhanced survival of transplants exposed to realistic stresses under field conditions. In "no stress" trials, the ABA treatments had little effect on yields.

c) showed that foliar or root applied ABA analogs can be used to "hold" plants at specific growth stages, thereby allowing their storage and/or extending the marketing period for specific sizes or stages of crop growth.

d) investigated the potential for using ABA analogues on potatoes to increase yields, alter tuber size distribution, increase tolerance to certain diseases and extend the natural dormancy period of the crop. Few consistent beneficial effects were found in three seasons of testing.

e) showed that the ABA analogs had little potential to be used as reversible sprouting inhibitors of stored potatoes.

e) demonstrated that the ABA analogs could be used to slow growth of amenity turf - thereby reducing maintenance and mowing costs.

Executive summary

Two synthetic analogues of the plant hormone Abscisic Acid (ABA) generated by researchers at PBI/NRC were evaluated in a series of trials for their potential to alter growth, physiology and stress tolerance of a range of high value Horticultural crops. The findings were;

a) ABA analogues slow water use by transplants of a range of horticultural crops (tomatoes, pumpkins, marigold, petunia). This reduction in water use could protect the seedlings against dessication stress that commonly occurs when seedlings are transplanted. The extent of the protection increased with the concentration of ABA applied. Applying the ABA as a root dip was more convenient and effective than foliar application, but utilized more chemical. The ABA analogues occasionally produced some dosage dependant phytotoxicity but treatment concentrations which were both effective and safe were easily determined.

b) Application of ABA analogues did not provide a significant degree of frost protection to transplants of the horticultural crops tested. However, the analogs did protect the seedlings against injury caused by chilling temperatures (0-5C).

c) treatment with the ABA analogues may be useful as a means for slowing the growth of seedlings. This was demonstrated using several species of ornamental annual flowers. In these crops, root treatment effectively stopped growth for a of two weeks or more - allowing the crop to be handled, stored or marketed at an ideal size.

d) when ABA treated seedlings of tomatoes (1998-2000), pumpkins (1998 and 2000) and ornamentals such as geraniums (1998) or impatiens (1999) were grown in the field in the absence of any significant stress, the treatments caused some dosage related delays in plant growth and maturity. In subsequent studies, dosages were identified that provided effective drought stress protection without any longterm negative impact on growth or yields.

e) foliar applications of ABA were evaluated for their potential to alter growth, tuber set and tuber dormancy in potatoes. ABA analogues were applied on three occasions (July, August, September) to Shepody potatoes. The ABA treatments had no impact on growth, tuber size distribution or yields.

f) preliminary studies indicated that foliar applied ABA had the potential to reduce Common Scab in potatoes. This response is likely related to the ABA slowing crop water use, thereby causing changes in the soil water balance unfavorable to scab infection. Foliar applications of ABA analogues were tested for their potential to control scab in Norland potatoes during 1998. None of the treatments significantly reduced the incidence or severity of scab. However, for cv. Shepody, foliar application of both PBI 365 and PBI 429 substantially improved yields relative to the control.

g) The impact of the applying ABA analogs to potatoes prior to storage was investigated. ABA is known to be involved in the onset and maintenance of dormancy. Although it was hoped that the ABA analogs might act as a reversible inhibitor of sprouting, no response to the ABA treatments was observed. .

h) a series of studies showed that foliar applied ABA analogs will slow growth of amenity turf with no negative impact on its appearance or longterm health. The economics of this treatment appear questionable unless more effective means of application are found.

In summary, this project identified several potential applications of the ABA analogues. Crucial steps were taken towards selecting the best ABA form and developing appropriate dosages and application techniques. Some anticipated ABA responses have not been confirmed - but other unanticipated uses have come to light.

Project Background

Horticultural crops are highly sensitive to environmental stresses such as extremes of temperature and moisture. Plants are particularly susceptible and sensitive to these stresses during transplanting and early establishment. Field conditions during the spring in Saskatchewan are frequently challenging for production of horticultural commodities, with chilling damage and drought stress, the most common problems.

The hormone ABA is known to be involved in plants stress physiology. This hormone acts as a signaling molecule in various physiological processes including responses to environmental stresses such as drought, cold, salinity and heat (Abrams, 1999). ABA seems to coordinate the overall physiology of plants either by suppressing or promoting processes depending on the circumstances (Addicott, 1983). ABA influences plants' drought and chilling response mechanisms by reducing transpiration water loss through stomatal closure. ABA also regulates the expression of the drought-induced genes (lea type) involved in plants' adaptation to environmental stresses (Bray, 1997).

Levels of ABA in plants can be increased through exogenous application. Treatment with can protect crops from desiccation by reducing transpirational water loss during drought stress (Abrams, 1999). Exogenous application of ABA significantly increased stomatal resistance and water potential of pepper seedlings thus reducing transplant shock in greenhouse and field experiments (Berkowitz and Rabin, 1989). Similarly application of ABA to the cut ends of leaf petioles of *Phaseolus vulgaris* and sugarbeet caused rapid stomatal closure within eight to nine minutes (Kriedemann, 1972). Stomatal water loss at transplanting was significantly reduced bare- rooted Brussels sprouts seedlings treated with ABA (Mc-Kee, 1978). Increased freezing tolerance, low temperature growth and advanced flowering were observed in ABA treated *Brassica napus* and *Brassica compestris* seedlings (Wilen et al. 1994).

The protective effects of exogenously applied ABA are very short lived due, in part, to poor uptake and rapid turn over in the plant systems (Abrams *et al*. 1997). To address this problem, chemically synthesized analogs of ABA have been developed which mimic the effects of ABA (Abrams *et al.* 1997). Alteration in the ABA structure cause the analogs to be metabolized more slowly than ABA, resulting in longer lasting and more effective biological activity (Abrams *et al.* 1997). Thus, ABA analogues have potential as crop protection chemicals, particularly for drought stress. The antitranspirant effects that the ABA analogues produced on barley leaves and water stressed cotton plants were found comparable with that of ABA (Carbonmier *et al*. 1981). Grossnikel *et al*. (1996) reported improved survival of ABA analog treated spruce seedlings under conditions of water stress in the field. ABA analogues were found to increase chilling as well as freezing resistance in a number of crops plants including maize, wheat, rice, cotton, cucumber (Dorffling *et al.* 1989). It appears that the ABA analogs trigger stomatal closure, which reduced water loss during chilling period. Similarly, a number of ABA analogs applied as a root drench to rye seedlings enhanced freezing tolerance and improved root growth (Abrams, 1999).

This research program was designed to test several practical applications of the ABA analogs in high-value Horticultural crops. Specifically, this project;

a) evaluated the efficacy of two of the most promising ABA analogs in alleviating cold and drought stress in high-value transplanted Horticultural crops. The study sought to determine optimum dosages and time of application, to quantify the effective duration of protection and the potential to use the ABA analogues enhance yield and profitability while reducing production risks

b) determined if the ABA analogues could be used to manage water use in crops such as potatoes -

thereby increasing yields, altering tuber size distribution and increasing tolerance to certain diseases.

c) determined if ABA could be used to "hold" plants at specific growth stages, thereby allowing their storage and/or extending the marketing period for specific sizes or stages of crop growth.

d) if ABA analogs could be used to control sprouting of stored potatoes in a reversible manner.

e) if ABA analogs could be used to slow growth of amenity turf - thereby reducing maintenance and mowing costs.

ABA Analogues and Stress Tolerance of Horticultural Crops - ADF Project 97000289

Section 1. Influence of ABA Analogues on Stress Tolerance of Horticultural Transplants

Trial # 1.1. Dosage response study for pre-transplanting root dips

Background

When utilizing exogenous application of hormones to govern plant growth, the application of a correct dosage is critical. The concentration of product applied and the method utilized to supply it must deliver a dosage sufficient to produce the desired effect for the appropriate duration of time. Insufficient dosages are ineffective, while excessive levels may be phytotoxic and economically inefficient.

Researchers working with ABA have found that application of this hormone as a root zone treatment is more effective (degree and duration of response) than foliar application. Treatment of transplants with ABA prior to moving to the field should enhance the transplants' ability to tolerate the stresses commonly encountered at this time (drought, heat or cold).

Objective: ***To evaluate stress tolerance of cucurbit transplants treated with various concentrations of the ABA analogue PBI 365 as a pre-transplanting root dip.***

Pumpkins were selected as the test crop as the grow rapidly and are very sensitive to drought stress following transplanting. Pumpkin seeds (*Cucurbita pepo* cv. Jack-o-Lantern) were introduced into Jiffy 7 peat pots on January 27 1997. Once germination was completed, the peat pots were moved into the Department of plant Sciences greenhouse (23°C/18°C, day/night), 12 h daylength with no supplemental lights. On February 28, the plants were treated with varying concentrations of the ABA analogue PBI 365 (provided by S. Abrams, PBI). At this point the plants had reached the developmental stage where pumpkins are typically transplanted into the field.
The treatments were;

Water + Solvent
10^{-3}, 10^{-4}, 10^{-5} M ABA 365 + Solvent

The rates were derived from past experience with plant responses to the ABA analogues (S. Abrams pers comm). The 10^{-5} rate represents a minimum concentration where responses could be expected while the 10^{-3} concentration represents a potentially phytotoxic level.

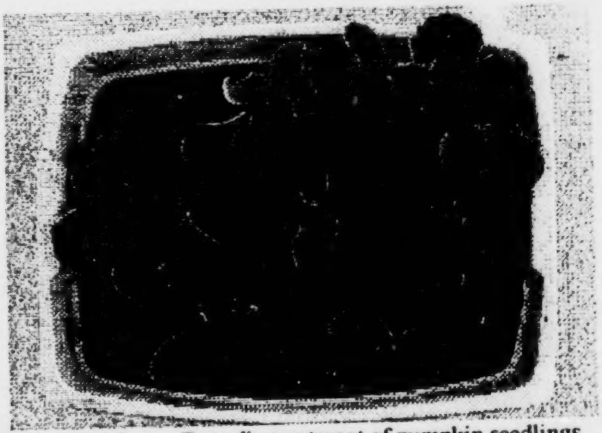


Figure 1.1.1. Root dip treatment of pumpkin seedlings

Plants were immersed in 1500 mL of the solution for 4 minutes (Figure 1.1.1). The amount of ABA solution remaining after the root dip treatment was measured. This allowed the exact amount applied to each plant to be determined. The amount absorbed per cell ranged from 35.3 to 32.5 mL with an average of 33.8 mL. Following treatment, the plants were returned to the greenhouse and grown out under the previously described conditions. One group of plants was watered as required over the next 10 days. This group was used to determine the impact of the ABA treatments on growth under non-stressed conditions. Water was withheld from the other group and the time to wilting was recorded for the various treatments. There were four replicates of each treatment and the treatments were arranged in a randomized complete block design of the greenhouse bench in order to correct for any gradients within the greenhouse. Each treatment replicated consisted of five plants in the stress trial and four plants in the non-stressed trial. On March 10, the experiment was terminated as all plants in the stressed trial had wilted. A range of growth parameters were examined for the plants in the non-stressed trial (number of leaves, fresh weight of the tops).

Results

There were no visual indications of any acute effects of the ABA dip. As time passed, the cotyledon leaves of most plants senesced. In some cases, the senescence spread to the primary leaves. This problems was most obvious in the non-stressed trial and in plants treated with the highest concentration of PBI 365 (see Figure 1.1.2).

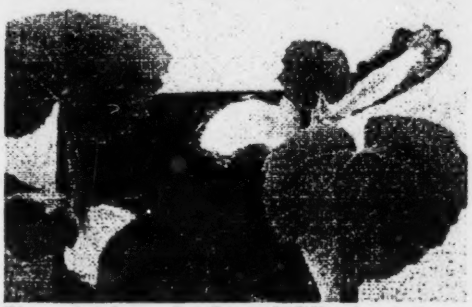


Figure 1.1.2. Leaf scorch following root dip treatment of pumpkin seedlings with ABA 365.

Stressed Trial.

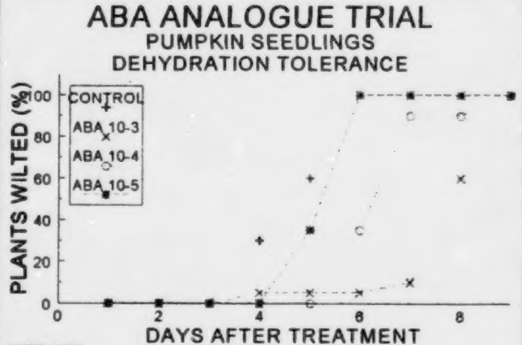


Fig. 1.1.3 Influence of ABA treatments on time to wilting of pumpkin seedlings

As indicated by Figure 1.1.3, treating pumpkin seedlings with PBI 365 as a root dip significantly slowed water loss, thereby increasing the plants' tolerance to water deficits. The time to wilting was positively correlated with the concentration of PBI 365 applied. The differences in time to wilting for the various treatments were relatively small (1-3 days). This reflects the large size of the plants relative to the moisture storage capacity of the Jiffy 7 pellets.

Non-Stressed Trial.
As indicated by Figure 1.1.4, the PBI 365 at 10^{-3} and 10^{-4}M reduced leaf numbers relative to the non-treated control when the plants were kept adequately watered throughout the trial. The 10^{-3} treatment also reduced the fresh weight of the tops relative to the controls (Figure 1.1.5). However, the 10^{-4} and 10^{-5} M treatments actually significantly increased top growth relative to the controls.

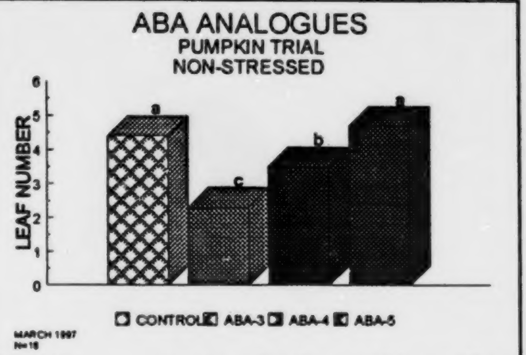


Fig. 1.1.4 Leaf number for pumpkin seedlings treated with varying concentrations of ABA 365

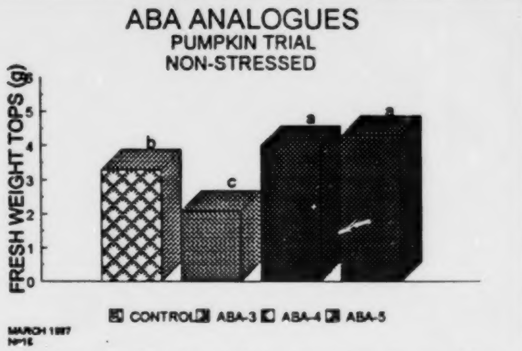


Figure 1.1.5. Fresh weights at harvest of pumpkin seedlings treated with varying concentrations of ABA 365

Conclusion -
Root dips containing PBI 365 were effective at slowing moisture loss by pumpkin seedlings. This suggests the potential for using PBI 365 to prevent/reduce transplant stress. Effectiveness of the ABA, as indicated by the rate of moisture loss, was dosage dependant. At the highest concentrations, the PBI 365 appeared to be somewhat phytotoxic as indicated by accelerated leaf senescence and reduced top growth. Low concentrations of ABA 365 applied to plants growing without stress actually seemed to enhance growth.

Recommendations - the trial be repeated with minimal modifications in order to confirm the results reported above.

Trial #1.2. Pre-transplanting Root Dips

Backgound

Trial 1.1 indicated that application of the ABA analogue PBI 365 as a root dip slowed moisture use by pumpkin seedlings. This should increase their tolerance of the drought stress commonly encountered at transplanting. The ABA effects were dosage dependant. Under non-stressed conditions, high concentrations of ABA caused symptoms indicative of senescence. Since ABA is involved in plant senescence, this dosage response was a concern. Trial 1.2 was designed to confirm the results in trial 1.1. The amounts of ABA applied via root drenching are substantial - this raises concerns about the economic viability of the treatments. Foliar application of chemicals is typically more efficient in terms of the amount of product required - but in most studies foliar application of ABA has been relatively ineffective. Trial 1.2 was designed to compare foliar versus root zone treatment with ABA.

Objective: ***To evaluate stress tolerance of cucurbit transplants treated with various rates of ABA analogue PBI 365 as a pre-transplanting root dip or via foliar application.***

Procedure:

Pumpkin seeds (*Cucurbita pepo* cv. Jack-o-Lantern) were introduced into Jiffy 7 peat pots on February 15. Once germination was completed, the peat pots were moved into the Department of Plant Sciences greenhouse (23°C/18°C, day/night), 14 h daylength with no supplemental lights. On April 18, the plants were treated with the appropriate concentration of the ABA analogue PBI 365 (provided by S. Abrams, PBI). Again, the plants at this time were at the stage where they are typically planted into the field.

The treatments were;

Water + Solvent
10^{-3}, 10^{-4}, 10^{-5} M ABA 365 + Solvent

Treatments were applied as a root dip as in trial # 1.1 and also as a foliar spray. In the root dip treatments, the plants were immersed in 1500 mL of the solution for 4 minutes. In the previous trial, the plants absorbed 35.3 to 32.5 mL per cell with an average of 33.8 mL. In the foliar applied treatments an atomizer was used to apply the spray to run off. No spreader/sticker was used. Following treatment, the surface of each Jiffy pot was covered with a 1 cm layer of perlite. The objective of this treatment was to eliminate evaporation as an avenue for moisture loss from the pots; thereby restricting moisture loss to transpiration. The plants were returned to the greenhouse and grown out under the previously described conditions. One group of plants for both the foliar and root dip treatments was watered as required over the next several weeks. This group was used to determine the impact of the ABA treatments under non-stressed conditions. Water was withheld from the other group and the time to wilting was recorded for the various treatments. There were four replicates of each treatment and the treatments were arranged in a randomized complete block design of the greenhouse bench in order to correct for any gradients within the greenhouse. Each treatment replicated consisted of five plants in the stress trial and four plants in the non-stressed trial. On May 5, the experiment was terminated as all plants in the stressed trial had wilted and plants in the non-stressed trial were becoming too large for the pots. A range of growth parameters were examined for the plants in the non-stressed trial (number of leaves, fresh weight of the tops).

Results

The foliar treatment used far less chemical than the root dip (10 ml/plant versus 30 ml/plant). As the foliar treatments ABA solutions were applied to run off there was some wastage that could be eliminated.

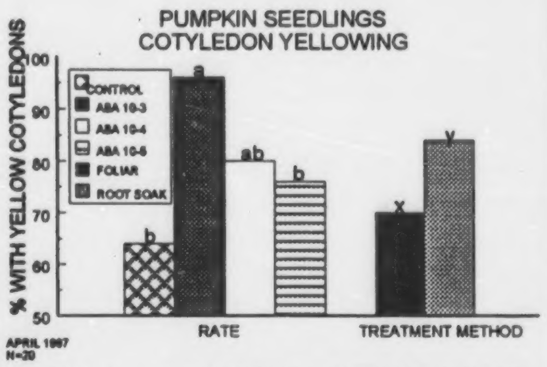


Figure 1.2.1 Leaf yellowing of pumpkin seedlings receiving varying rates of ABA 365 as a root drench or via foliar application.

In trial #1.1, there were few visual indications of any acute effects of the ABA dip. As time passed, the cotyledon leaves of most plants had senesced. In some cases, the senescence spread to the primary leaves. This problems was most obvious in the non-stressed trial and in plants treated with the highest concentration of ABA 365. Similar but more severe symptoms were observed in trial 1.2 (Figure 1.2.1). The root soak treatments showed more yellowing than the foliar treated plants.

Stressed Trial.

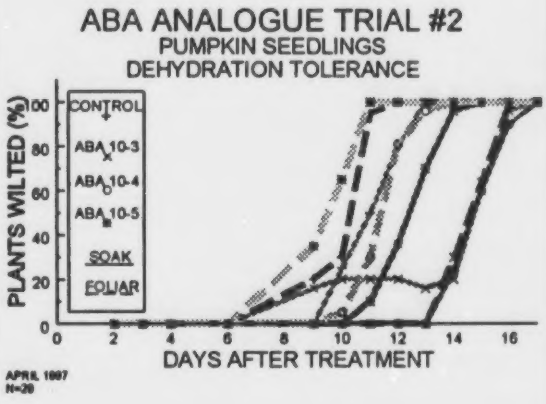


Figure 1.1.2 Time to wilting for pumpkin seedlings treated with ABA 365 as a root dip or via foliar spray.

As indicated by Figure 1.2.2, treating pumpkin seedlings with ABA 365 as either a root dip or through foliar application significantly slowed water loss, thereby increasing the plants' tolerance to water deficits. The time to wilting was again positively correlated with the concentration of ABA 365 applied. The differences in time to wilting were somewhat larger than in trial 1.1. This can be attributed to the use of perlite as a means of preventing evaporative moisture loss from the pots.

The root dip treatment was more effective at each concentration than the corresponding foliar treatment. However, given that the root dip treatment involved the application of much more of the active ingredient, foliar treatment would appear to be a promising option in terms of cost-efficiency. Foliar treatment at higher concentrations or multiple foliar applications may be worth examining.

Non-Stressed Trial.

Pumpkin seedlings developed a significant degree of chlorosis during the observation period. The cause of the chlorosis could not be determined. The extent of the chlorosis was not related to the ABA treatments. As indicated by Figure 1.2.3, application of PBI 365 had a dosage dependant inhibitory effect on growth of the pumpkin seedlings under non-stressed conditions. Where the ABA was applied to the foliage, only the most concentrated solution (10^{-3} M) produced a lasting negative effect on growth. Both the 10-3 and the 10^{-4}M treatments reduced growth when applied as a root soak.

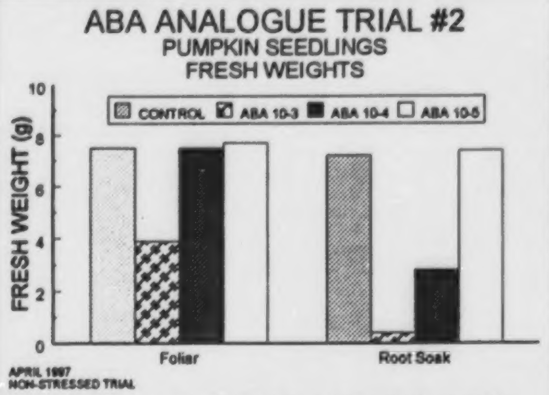


Figure 1.2.3 Fresh weight at harvest of pumpkin seedlings treated with ABA 365 as a foliar or root drench treatment.

Conclusion -

Application of PBI 365 was again effective at slowing moisture loss by pumpkin seedlings. This suggests the potential for using PBI 365 to prevent/reduce transplant stress. Effectiveness of the ABA, as indicated by the rate of moisture loss, was dosage dependant. At the highest concentrations, the PBI 365 appeared to be somewhat phytotoxic as indicated by accelerated leaf senescence and reduced top growth. The root dip treatment was more effective at each concentration than the corresponding foliar treatment. However, given that the root dip treatment involved the application of much more of the active ingredient, foliar treatment would appear to be a promising option in terms of cost-efficiency. Foliar treatment at higher concentrations or multiple foliar applications may be worth examining.

Trial 1.3. Pre-transplanting Root Dips for Tomato Seedlings

Background

Trials 1.1 and 1.2 had indicated that application of the ABA analogue PBI 365 as a root dip slowed moisture use by pumpkin seedlings. This should increase their tolerance of the drought stress commonly encountered at transplanting. The ABA effects were dosage dependant. Under non-stressed conditions, high concentrations of ABA slowed growth and caused symptoms indicative of senescence. Since ABA is involved in plant senescence this dosage response was a concern. In Trial 1.2, foliar application of ABA was shown to be less effective than root dips but the foliar application required a fraction of the chemical involved in the root dips. To confirm the responses observed using pumpkins as the test crop, trial 1.2 was repeated utilizing tomatoes. Tomatoes represent another commonly transplanted Horticultural crop but tomatoes exhibit fundamentally different growth habit and management response characteristics than pumpkins. Tomatoes are more resistant to dehydration than pumpkins and their narrow, hairy leaves make them difficult to treat with foliar applied chemicals.

Objective: ***To evaluate stress tolerance of tomato transplants treated with various rates of the ABA analogue PBI 365 as a pre-transplanting root dip or via foliar application.***

Procedure:

Tomatoes (*Solanum esculenta* cv. Spitfire) were seeded into 85 cm^3 cell packs containing Sunshine Mix 4 on April 1. The plants were grown out in the Department of Plant Sciences greenhouse (23°C/18°C, day/night), 14 h daylength with no supplemental lights. On April 28 the plants were treated with the appropriate concentration of the ABA analogue PBI 365 (provided by S. Abrams, PBI). Tomatoes are typically transplanted at about this developmental stage (4 weeks).

The treatments were;

Water + Solvent
10^{-3}, 10^{-4}, 10^{-5} M PBI 365 + Solvent

Treatments were applied as a root dip as in Trial 1.1 and also as a foliar spray as in Trial 1.2. In the root dip treatments, the plants were immersed in the appropriate concentration of ABA solution for 4 minutes. In previous trials plants absorbed 35.3 to 32.5 mL per cell with an average of 33.8 mL. In the foliar applied treatments, an atomizer was used to apply the spray to leaf run off . Again no spreader/sticker was used in the foliar application trials. Following the ABA treatments, the surface of each pot was covered with a 1 cm layer of perlite. The perlite eliminated evaporation as an avenue for moisture loss from the pots; thereby restricting moisture loss to transpiration. The plants were returned to the greenhouse and grown out under the previously described conditions. One group of plants for both the foliar and root dip treatments was watered as required over the next several weeks. This group was used to determine the impact of the ABA treatments under non-stressed conditions. Water was withheld from the other group and the time to wilting was recorded for the various treatments. There were four replicates of each treatment and the treatments were arranged in a randomized complete block design of the greenhouse bench in order to correct for any gradients within the greenhouse. Each treatment replicated consisted of six plants. On May 10 the stressed trial was terminated as all plants had wilted. The non-stressed trial was continued until May 25. A range of growth parameters were examined for the plants in the non-stressed trial (number of leaves, fresh weight of the tops).

Results

Crop health was excellent throughout this trial.

Stressed Trial.

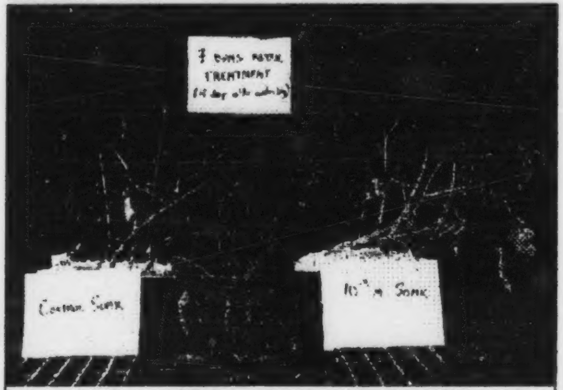


Figure 1.3.1 ABA treatments delaying wilting of tomato seedlings.

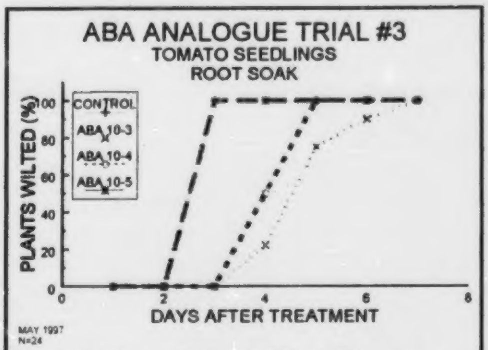


Figure 1.3.2. Rate of wilting of tomato seedlings treated with ABA 365 as a root drench

The plants were at a relatively advanced stage of development when treated and conditions in the greenhouse were very warm, with abundant sunlight. As a consequence, water use rates were high. Control plants wilted within two days of the cessation of watering (Figure 1.3.1) as did all the plants treated with PBI 365 via the foliar spray. Application of PBI 365 as a root dip at 10-3 and 10-4 M significantly increased the period of time prior to wilting (Figure 1.3.2).

Non-stressed trial

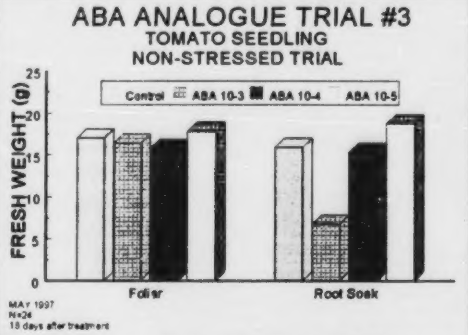


Fig. 1.3.3 Impact of root and foliar applied ABA 365 of growth of tomato seedlings.

The ABA treatments caused no obvious changes in plant morphology or development under non-stressed conditions. Foliar application of ABA had no negative impact on crop growth (fresh weights) under non-stressed conditions (Figure 1.3.3). The 10-3 M root dip treatment did result in a significant reduction in plant growth.

Conclusion - root dips containing the ABA analogue PBI 365 can slow water use of tomato transplants thereby potentially increasing drought tress tolerance. Foliar application of ABA to tomatoes was not as effective as root dips. This is not surprising as the amount of chemical supplied to each plant via foliar application is a small fraction of the amount applied in the root dips. Pumpkins showed a greater response to the foliar application of ABA than the tomatoes. This may reflect species to species differences in sensitivity or the wide flat leaves of the pumpkins may have captured more of the foliar applied product.

Trial 1.4. ABA as a Pre-transplanting Treatment for Tomato Seedlings

Background

Previous research (Trial 1.3) indicated that, for tomatoes, application of ABA 365 as a root drench was more effective than foliar application of a given concentration of ABA analogue. In pumpkins, foliar treatment was as effective as root drenches. Foliar treatments have the advantages of requiring less chemical than root drench treatments. **This trial was designed to confirm the previous findings on the relative efficacy of foliar versus root treatment of tomato seedlings. ABA 429 was included as an alternative to ABA 365.**

Objective: ***To evaluate stress tolerance and growth patterns of tomato transplants treated with various rates of the ABA analogue PBI 365 and 429 as a pre-transplanting root dip or via foliar application.***

Procedure:

Tomatoes (*Solanum esculenta* cv. Spitfire) were seeded into 85 cm^3 cell packs containing Sunshine Mix 4 on March 1, 1999 . The plants were grown out in the Department of Plant Sciences greenhouse (23°C/18°C, day/night), 14 h daylength with no supplemental lights. Once the plants reached the four leaf stage (April 6), the plants were treated with the appropriate concentration of the ABA analogue PBI 365 or PBI 429 (provided by S. Abrams, PBI). Tomatoes are typically transplanted at about this developmental stage (4 weeks).

The treatments were;

10^{-3} or 10^{-4} M PBI 365 or PBI 429 + Solvent as a foliar application
10^{-4} M PBI 365 or PBI 429 + Solvent as a root drench
Control = Water + Solvent applied as either a root drench or foliar

Root dip procedures were outlined in Trial 1.1, while the foliar application technique was outlined in Trial 1.2. In the root dip treatments, the plants were immersed in the appropriate concentration of ABA solution for 4 minutes. In previous trials, the root drench used 35.3 to 32.5 mL per cell with an average of 33.8 mL. In the foliar applied treatments an atomizer was used to apply the spray to leaf run off. The volume of spray required to treat one plant was about 5.4 mL. Tween 20 (0.1% v/v) was used as a spreader/sticker in the foliar application trials. The plants were returned to the greenhouse and grown out under the previously described conditions. One group of plants for both the foliar and root dip treatments was watered as required over the next several weeks; this group was used to determine the impact of the ABA treatments under non-stressed conditions. The incidence of leaf yellowing was recorded in the non-stressed treatment, using a scale of 0= no yellowing to 5 = severe yellowing of all leaves. Water was withheld from the other group and the severity of wilting was recorded daily using a scale of 0 = no wilting to 5 = dead. There were four replicates of each treatment and the treatments were arranged in a randomized complete block design of the greenhouse bench in order to correct for any gradients within the greenhouse. Each treatment replicated consisted of four plants. On April 17, (11 days after treatment) the stress trial was terminated as all plants had wilted. The non-stressed trial was terminated May 2, at which time the plants' growth characteristics (fresh weight and height of the tops, stem thickness, flower and fruit numbers) were measured.

Results

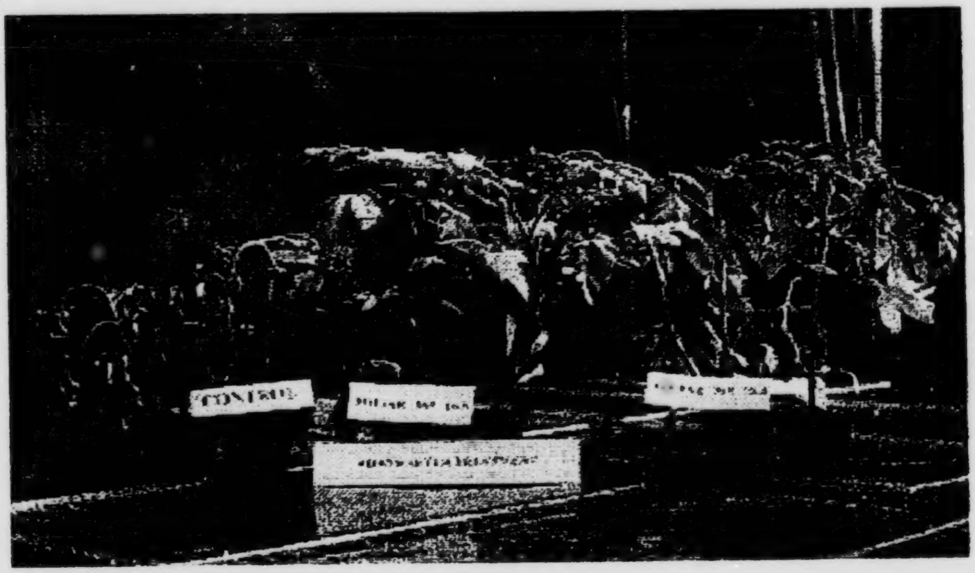


Figure 1.4..1. Influence of a foliar applied ABA analog (PBI 365) on drought stress tolerance of tomato seedlings (4 days without water)

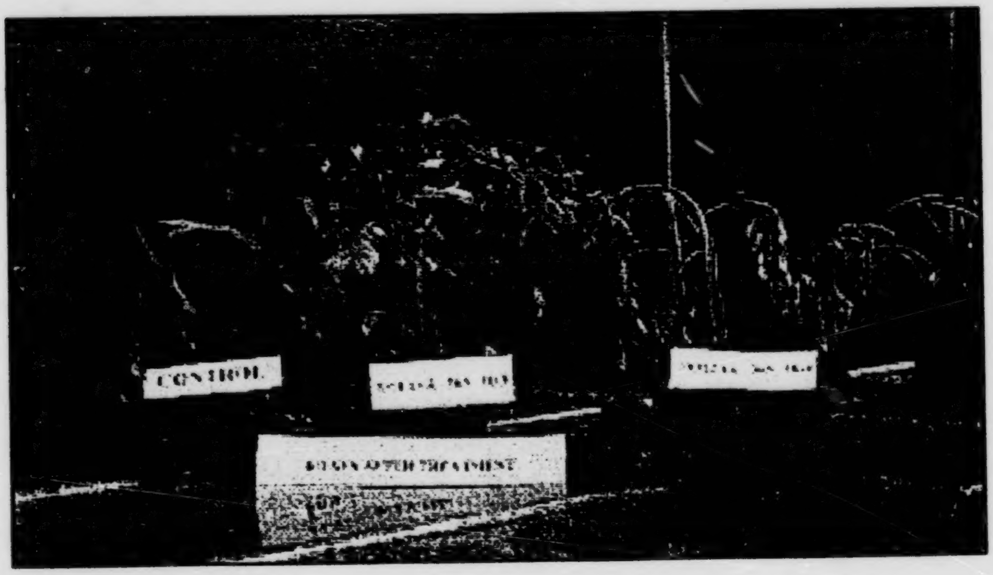


Figure 1.4.2. Influence of a foliar applied ABA analog (PBI 365) on drought stress tolerance of tomato seedlings (6 days without water)

of tomato seedlings (6 days without water)

Stressed Trial.

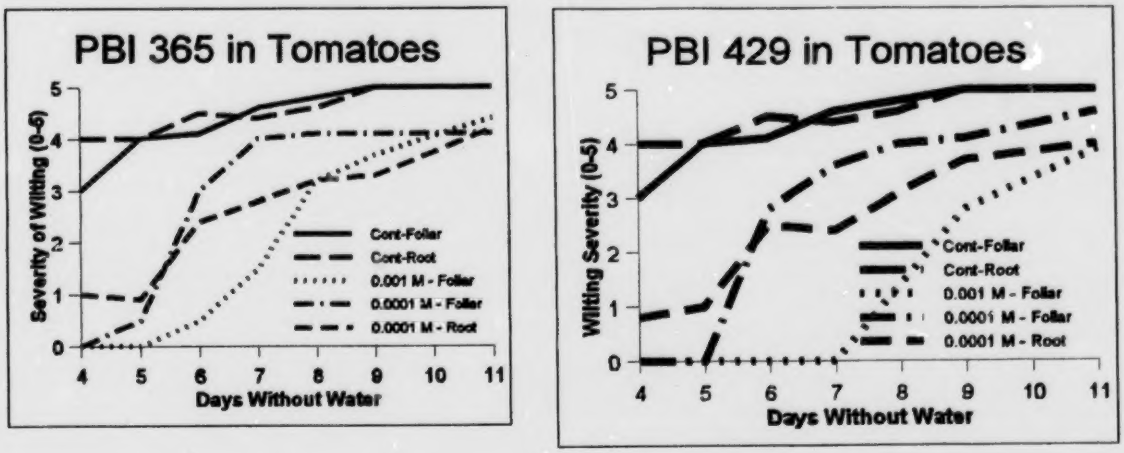
Figures 1.4.1 and 1.4.2 illustrate the plant responses to foliar application of two concentrations of PBI 365. The plants were at a relatively advanced stage of development when treated and conditions in the greenhouse were warm, with abundant sunlight. As a consequence, water use rates were high. Control plants wilted within 4-5 days of the cessation of watering (Table 1.4.1 and Figure 1.4.3). The two ABA analogues had relatively similar effects on drought stress tolerance of the tomato seedlings, irrespective of whether the ABA was applied as a foliar spray or as a root drench. When applied as a root drench, 10-4 M ABA 365 and ABA 429 delayed wilting for several days (Figure 1.4.3). For the first few days after treatment, the 10-4 M foliar treatment was quite effective at slowing water use and wilting (Figure 1.4.3). From day 0-6, the 10-4 M foliar treatment was actually more effective in reducing plant stress than the equivalent concentration of ABA applied as a root drench. However, after day 6, the foliar treated plants lost water rapidly, while the plants treated with the root drench lost water more slowly. Foliar application of ABA 365 and ABA 429 at 10-3 M was highly effective in delaying wilting, with some plants surviving 11 days without water (Figure 1.4.3).

Table 1.4.1. Severity of wilting for tomato seedlings at various intervals after treatment with ABA analogues by foliar or root drench applications.

	DAYS AFTER TREATMENT						
	4	5	6	7	8	9	11
Control Root	4a	4a	4.5a	4.4a	4.6ab	5.0a	5.0a
Control Foliar	3b	4a	4.1a	4.6a	4.8a	5.0a	5.0a
365x10^{-3} Foliar	0_E	0d	0.5c	1.5d	3.2d	3.7c	4.4b
365x10^{-4} Foliar	0_E	0.3b	0.5c	4.0ab	4.1bc	4.1b	5.0a
429x10^{-3} Foliar	0_E	0d	0c	0e	1.4e	2.8e	3.9d
429x10^{-4} Foliar	0_E	0d	2.8b	3.6b	4.0c	4.1b	4.6b
365x10^{-4} Root	1c	0.9c	2.4b	2.8c	3.2d	3.3d	4.2c
429x10^{-4} Root	0.8d	1c	2.5b	2.4c	3.1d	3.7c	4.0c
LSD (0.05)	**0.12**	**0.05**	**0.5**	**0.66**	**0.51**	**0.25**	**0.22**

0 = no wilting, 5=complete wilting

Figure 1.4.3. Wilting of tomato seedlings treated with ABA analogs (PBI 365 and PBI 429) as a root drench or via foliar application.



Non-stressed trial

In previous trials, some yellowing of leaves had been noted following application of the ABA analogues. Some yellowing of the lower leaves was also noted in this trial, starting at 5 days after treatment (Table 1.4.2). The extent of yellowing did not change over time (Table 1.4.2). As a general rule, yellowing was more noted;

a) in root applied versus foliar treated plants,
b) with the higher rates of analogue and
c) with PBI 429 versus PBI 365.

It is noteworthy that the control plants in the root drench treatments showed relatively high levels of yellowing. This suggests that the solvent used to dissolve the ABA may be the actual cause of the yellowing. There was minimal yellowing for the control treatment in the foliar sprayed trial. The ABA treatments caused few obvious changes in plant morphology although growth was clearly slowed. One noteworthy observation is that the **foliar applied ABA appear to promote early flowering**. (Table 1.4.2). Both analogues and both treatment rates had this effect. Root applied ABA did not promote flowering.

Table 1.4.2. Degree of leaf yellowing and flowering of tomato seedlings after treatment with ABA analogues via foliar or root drench application.

	YELLOWING (0-5)				Flowering (%)	
	Day 5	**Day 8**	**Day 11**	**Day 18**	**Day 11**	**Day 18**
Control Root	2.06ab	2.38a	1.83b	1.67b	0	8%
Control Foliar	0.58e	0.33d	0.40d	0.75	0	25%b
365x10^{-3} Foliar	1.33cd	1.25c	1.41bc	1.25bc	75%a	75%a
365x10^{-4} Foliar	1.16d	1.08c	0.75cd	0.83cd	33%b	100%a
429x10^{-3} Foliar	2.07ab	2.08ab	2.08ab	2.41a	25%	25%b
429x10^{-4} Foliar	1.92ab	1.50bc	2.08ab	1.67b	58%a	75%a
365x10^{-4} Root	1.75bc	2.00ab	2.21ab	2.41a	0	8%
429x10^{-4} Root	2.41a	2.41a	2.75a	2.75a	0	0%
LSD (0.05)	***0.51***	***0.61***	***0.78***	***0.50***	***25%***	***32%***

At the final harvest, ABA effects on growth and development of the seedlings were determined (Table 1.4.3). All the vegetative growth parameters measured (height, weight and stem thickness) showed similar treatment effects as illustrated by the Fresh weight data presented in Figure 1.4.4;

a) the ABA treatments reduced growth relative to the controls.
b) the degree of growth inhibition increased with the concentration of ABA applied
c) ABA applied as a root drench had a greater negative effect on plant growth than a corresponding rate of ABA applied to the foliage.
d) the ABA treatments had relatively little effect on reproductive structures (blossom number, fruit number and size).

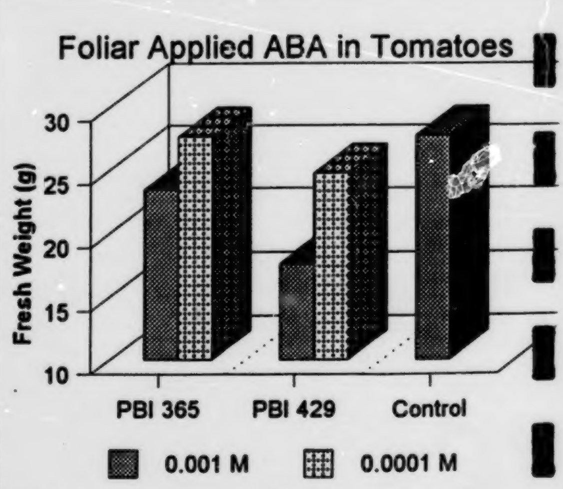


Fig. 1.4.4. Influence of foliar applied ABA on growth of tomato seedlings

Table 1.4. 3. Growth characteristics of tomato seedling 28 days after treatment with ABA analogues via foliar or root drench application.

	Fresh Weight (g)	Dry Matter (%)	Height (cm)	Stem width (mm)	Blossom #	Fruit #	Fruit Weight (g)
Control Root	28.6a	9.7a	37.5ab	6.5a	10.2a	0.2bc	0.8b
Control Foliar	26.6ab	9.7a	38.8a	5.7b	9.4ab	0.5ab	0.4b
365x10^{-3}	23.4cd	9.7a	35.5bc	6.3ab	8.5abc	0.2bc	0.8
x10^{-4}	27.6a	10.6a	33.5cd	6.9a	7.5cde	0.8a	2.7a
429x10^{-3}	17.6e	10.4a	26.7e	6.7a	5.8d	0.1c	0.3b
x10^{-4}	24.7bc	10.9a	37.1ab	6.4ab	8.9abc	0.2bc	1.0b
365 Root (10^{-4})	21.9d	10.2a	32.3d	6.5a	9.7ab	0	0b
429 Root (10^{-4})	13.3f	12.2b	22.6f	6.3ab	6.8cd	0	0b
LSD	2.7	1.1	2.7	0.7	2.3	0.4	1.6

Conclusion - the results confirm the previous findings that ABA analogues applied to seedlings as either a root drench or as a foliar spray can slow water use of tomato transplants thereby potentially increasing drought tress tolerance. In the previous trial, foliar applications of ABA were not as effective as root dips in controlling water use. This study suggests that foliar applications are as effective as root drenches but have a shorter duration of activity. This could be expected since the root drench supplies much more chemical than the foliar application. The peat moss could also act as a delayed release mechanism for the applied ABA. The limited period of activity of the foliar applied product could be advantageous in situations where only short-term effects are required or desired. Foliar application requires less chemical and less labor than root drenches - characteristics which would be desirable from a growers perspective. Foliar application of ABA appeared to accelerate flowering - this is the first time this effect has been observed in this research program. The merits of shortening the juvenile period in crops such as tomatoes are obvious, providing that earliness does not interfere with final yield potential.

The results of the non-stressed trial indicate that foliar and root applied ABA analogues have the potential to be used as "dwarfing" or "holding" agents for seedlings. The ABA reduced vegetative growth but did not effect physiological development. The degree of activity looks to be readily controlled through dosage, analogue type and method of delivery. As observed in the stressed trial, foliar application appears to be as effective as root drenches but with a shorter period of activity.

Trial 1.5. ABA as a Pre-transplanting Treatment for Pumpkin Seedlings

Background

Previous research (Trial 1.2,, 1.3 and 1.4) indicated that application of ABA as a root drench was more effective than foliar application at a given concentration of ABA analogue. However, using root drenches required much more chemical than did foliar treatment. Root applied ABA was also more frequently phytotoxic that foliar applied. In trial 1.4, ABA 429 was evaluated as an alternative to ABA 365 in tomatoes. This trial was designed to compare the two ABA analogues PBI 365 and 429 for foliar versus root application on pumpkins.

Objective: ***To evaluate stress tolerance and growth patterns of pumpkin transplants treated with various rates of the ABA analogue PBI 365 and 429 as a pre-transplanting root dip or via foliar application.***

Procedure:

Pumpkins (Curcurbita pepo cv. Jack O’’Lantern) were seeded into Jiffy 7s on April 5, 1999 . The plants were grown out in the Department of Plant Sciences greenhouse (23°C/18°C, day/night), 14 h daylength with no supplemental lights. Once the plants reached the two true leaf stage (April 20), the plants were treated with the appropriate concentration of the ABA analogue PBI 365 or PBI 429 (provided by S. Abrams, PBI). Pumpkins are typically transplanted at about this developmental stage (2 weeks).

The treatments were;

10^{-3} or 10^{-4} M PBI 365 or PBI 429 + Solvent as a foliar application
10^{-4} M PBI 365 or PBI 429 + Solvent as a root drench
Control = Water + Solvent applied as either a root drench or foliar

Root dip procedures were outlined in Trial 1.1, while the foliar application technique was outlined in Trial 1.2. In the root dip treatments, the plants were immersed in the appropriate concentration of ABA solution for 4 minutes. In previous trials plants absorbed 35.3 to 32.5 mL per cell with an average of 33.8 mL. In the foliar applied treatments an atomizer was used to apply the spray to leaf run off. The volume of spray required to treat one plant was about 9 mL. Tween (0.1% v/v) was used as a spreader/sticker in the foliar application trials. Following the ABA treatments, the surface of each pot was covered with a 1 cm layer of perlite. The perlite eliminated evaporation as an avenue for moisture loss from the pots; thereby restricting moisture loss to transpiration. The plants were returned to the greenhouse and grown out under the previously described conditions. One group of plants for both the foliar and root dip treatments was watered as required over the next several weeks. This group was used to determine the impact of the ABA treatments under non-stressed conditions. The incidence of leaf yellowing was recorded in the non-stressed treatment, using a scale of 0= no yellowing to 5 = severe yellowing of all leaves. Water was withheld from the other group and the severity of wilting was recorded daily using a scale of 0 = no wilting to 5 = dead. There were four replicates of each treatment and the treatments were arranged in a randomized complete block design of the greenhouse bench in order to correct for any gradients within the greenhouse. Each treatment replicated consisted of four plants. On May 9, (19 days after treatment) the stress trial was terminated as all plants had wilted. The non-stressed trial was terminated June 1 at which time the plants' growth characteristics (fresh weight and height of the tops, stem thickness, blossom number) were measured.

Results

Crop health was not exceptionally good in this trial. For reasons we were unable to determine, the control plants used water exceptionally quickly and were difficult to keep in a non-stressed state. This stress resulted in some necrosis to the cotyledon leaves.

Stressed Trial.

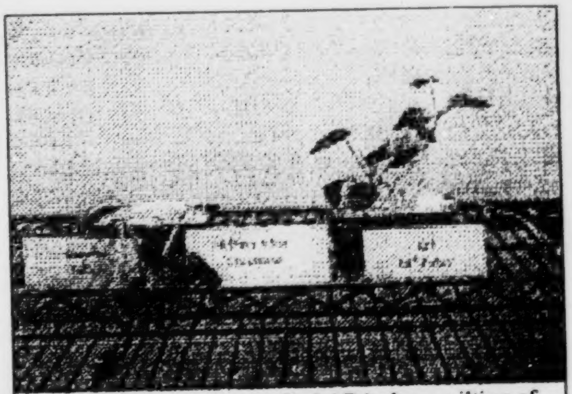


Figure 1.5.1 Foliar applied ABA slows wilting of pumpkin seedlings.

Figure 1.5.1 illustrates the pumpkin seedlings' response to foliar application of the ABA analogs. The plants were at a relatively advanced stage of development when treated and conditions in the greenhouse were warm, with abundant sunlight. As a consequence, water use rates were high. Control plants wilted within 4-6 days of the cessation of watering (Figure 1.5.1, Table 1.5.1 and Figure 1.5.2). Foliar application of the higher concentrations of both analogues was quite phytotoxic. Symptoms of this toxicity were apparent within 6 days of treatment and progressively worsened with time. Over 50% of the seedlings treated with PBI 429 at 10-3 M were dead by the end of the trial. The lower rate of both analogs did not adversely affect the seedlings' survival. Foliar application of both PBI 365 and 429 delayed wilting of the pumpkin seedlings relative to the controls. Effectiveness was concentration dependant, with the 10-3 M having much greater effects than the 10-4M. The analogs were relatively similar in terms of their efficacy as foliar treatments. When the ABA was applied as a root drench, PBI 365 was relatively ineffective. However, PBI 429 as a root drench was the slowest of all treatments to wilt - this efficacy came with none of the phytotoxicity associated with the more effective foliar treatments.

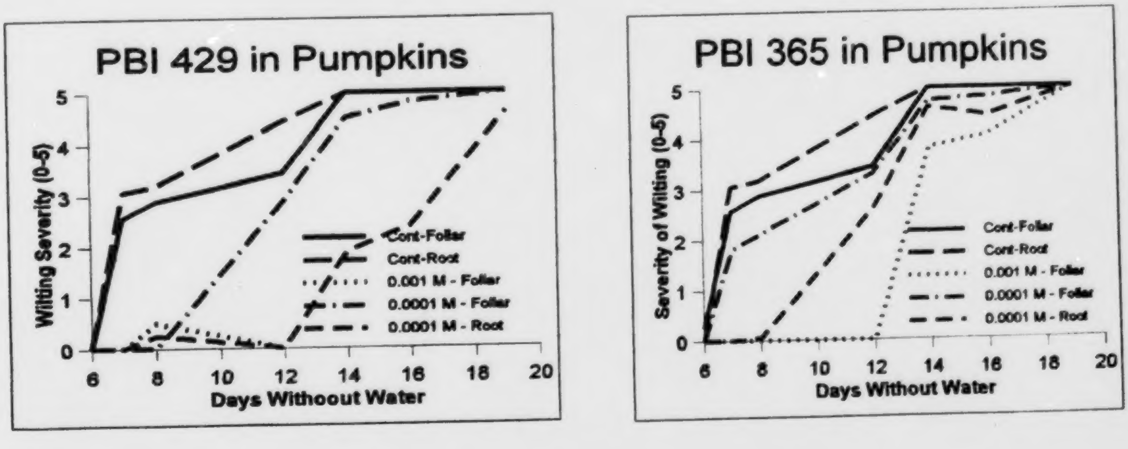


Figure 1.5.2. Wilting of pumpkin seedlings treated with ABA analogs (PBI 365 and PBI 429).

Table 1.5.1. Severity of wilting for pumpkin seedlings at various intervals after treatment with ABA

Table 1.5.1. Severity of wilting for pumpkin seedlings at various intervals after treatment with ABA analogues by foliar or root drench applications.

	DAYS AFTER TREATMENT						
	6	7	8	12	14	16	19
Control Root	0.25a	2.56ab	2.87ab	3.43b	5.0a	5.0a	5.0a
Control Foliar	0a	3.06a	3.18a	4.45a	5.0a	5.0a	5.0a
365x10^{-3} Foliar	0a	0c	0c	0d	3.81b	4.06c	5.0a
365x10^{-4} Foliar	0a	1.80b	2.12b	3.31bc	4.75a	4.81ab	5.0a
429x10^{-3} Foliar	0a	0c	0.5c	0d	***	***	***
429x10^{-4} Foliar	0a	0c	0c	2.88bc	4.5a	4.81ab	5.0a
365x10^{-4} Root	0a	0c	0.06c	2.62c	4.62a	4.43bc	5.0a
429x10^{-4} Root	0a	0c	0.25c	0d	1.88c	2.38d	4.6b
LSD (0.05)	**0.11**	**0.85**	**0.96**	**0.74**	**0.59**	**0.52**	**0.62**

Severity indices : 0=no wilting, 5= severe wilting, *** = plants dead

Non-stressed trial

In the stressed trial, treatment of the pumpkin seedlings with 0.001M PBI 365 or 429 caused some plants to die. Similar results were obtained for the non-stressed trial (Table 1.5.2). Beginning 5-6 days after treatment, the lower leaves of damaged plants began to yellow. The symptoms eventually spread to the entire plant. As noted for the tomatoes in trial 3.c, leaf yellowing and plant death was more noted;

a) in root applied versus foliar treated plants,
b) with the higher rates of analogue and
c) with PBI 429 versus PBI 365.

Table 1.5.2. Degree of leaf yellowing and average number of dead plants for pumpkin seedlings after treatment with ABA analogues via foliar or root drench application (non-stressed conditions).

	YELLOWING (0-5)		**Number of Dead Plants**	
	Day 6	**Day 7**	**Day 8**	**Day12**
Control Root	0d	0e	0c	0d
Control Foliar	0d	0e	0c	0d
365x10^{-3} Foliar	2.25a	2.50bc	1.25b	2.0b
365x10^{-4} Foliar	0d	0e	0c	0
429x10^{-3} Foliar	2.75a	4.00a	2.50a	3.75a
429x10^{-4} Foliar	1.50b	1.25cd	0c	0d
365x10^{-4} Root	0.5cd	0.50de	0c	0d
429x10^{-4}Root	1.0bc	1.50c	0c	0.25c
LSD	***0.56***	***0.78***	***0.71***	***0.82***

In the tomato trials, the control plants in the root drench treatments showed relatively high levels of yellowing. No such response was noted in the pumpkins. The ABA treatments caused few obvious changes in plant morphology in the non-stressed trial. Foliar application of PBI 429 at 10-3 M reduced growth of the seedlings (Table 1.5.3). Otherwise the ABA treatments (root and foliar applied) were comparable to the respective controls. As noted earlier, the control treatments experienced a degree of moisture stress at various intervals during the trial. This stress may have limited the growth of the controls relative to the ABA treated plants. The ABA treatments had no impact on flowering of the seedlings in the pumpkin trial - this does not correspond with the results obtained for the tomatoes where ABA appeared to promote flowering.

Table. 1.5.3. INFLUENCE OF ABA ANALOGS APPLIED AS FOLIAR OR ROOT DRENCH TREATMENTS TO PUMPKIN SEEDLING (NON-STRESSED TRIAL)

	Fresh Weight (g)	Dry Weight (g)	Blossom #
Control Foliar	5.07a	0.61a	4.0a
Control Root	4.70a	0.60a	5.25a
PBI 365 10^{-3}M	4.5a	0.53a	4.83a
PBI 365 10^{-4}M	5.07a	0.61a	4.92a
PBI 429 10^{-3M}	2.12b	0.24b	0b
PBI 429 10^{-4}M	4.67a	0.62a	4.68a
PBI 365 Root Drench	6.13a	0.62a	5.25a
PBI 429 Root Drench	-	-	-

Contrasts			
ABA Foliar vs. Control	p = 0.01	p = 0.01	p = 0.47
PBI 365 vs. 429	p = 0.0001	p = 0.01	p = 0.0001
0.001M vs. 0.0001M	p = 0.0001	p = 0.001	p = 0.0001
Root Drench vs. Foliar	p = 0.22	p = 0.01	p = 0.03
ABA Root vs. Control	p = 0.05	p = 0.70	p = 0.99
Interaction Analog * Rate	p = 0.0001	p = 0.001	p = 0.0001

Conclusion - the results confirm the previous findings that ABA analogues applied to seedlings as either a root drench or as a foliar spray can slow water use of pumpkin transplants thereby potentially increasing drought stress tolerance. In previous trials, foliar applications of ABA were not as effective as root dips in controlling water use. This study suggests that foliar applied ABA can be as effective as root drenches in slowing water use, particularly if the longer lasting for of ABA is used (ABA 429). The foliar application has the advantage of using less chemical but relatively concentrated solutions are required for maximum efficacy. Unfortunately, foliar application of highly concentrated ABA analogs produced severe phytoxicity in this trial. The symptoms of phytotoxicity - leaf yellowing, necrosis and senecsence began with the lower leaves present at the time of treatment and became progressively more severe and widespread as time passed after treatment. PBI 429 was more phytotoxic than PBI 365; This corresponds with the findings in previous trials. The treatment which provided the best combination of reduced water use without excessive phytotoxicity was PBI 429 applied as a root drench.

The results of the non-stressed trial indicate that foliar and root applied ABA analogues had only limited potential to be used as "dwarfing" or "holding" agents for pumpkin seedlings.

Trial 1.6. Confirmation Studies on the Impact of ABA Analogues on Drought Tolerance of Transplanted Horticultural Crops

Introduction

Previous studies had indicated the potential for using ABA analog applied as root drenches to slow water use of vegetable crop transplants. Root drenches were more effective than folair applied ABA and in some cases caused less phytotoxicity (Trial 1.5). The ABA effects were dosage dependant and analog specific as were problems with phytotoxicity.

Objective:

To determine the efficacy of 8' methylene and 8' acetylene ABA analogues in prevention of water stress in horticulture crop transplants.

Materials and Methods

Pumpkins (*Cucurbita pepo* cv Jack 'O Lantern) and tomatoes (*Solanum esculenta* cv Roadside Red) were planted into Jiffy 7's at a seeding rate of two seeds per pellet. The plants were grown in a greenhouse under day/night temperatures of 23°C/18°C with 12 hour day length. Fertilizer was applied at weekly intervals, through the addition of a liquid NPK 20-20-20. After one week, the crops were thinned to one plant per Jiffy 7. After two and one half weeks of growth, chemicals were applied. The average amount of the ABA analog solution absorbed by each seedling was 15.6 and 11.9 ml for the pumpkins and the tomatoes, respectively. The ABA analogues were applied at concentrations of 10^{-4} M and 5 X 10^{-5} M. Each treatment was applied to ten plants which were arranged into a complete random block design in a 50-cell tray. After chemical application, the crops were returned to the greenhouse and subjected to a drought stress treatment. The plants were held without water and the degree of wilting was measured daily on a scale of 0 to 5, where 0 represented full turgidity and 5 stood for completely wilted, dead plants. The experiment was terminated once one of the analogue treatments displayed greater than 50 percent wilting.

Results

In this study both test crops showed an increased tolerance to drought through the application of ABA analogues. Figures 1.6.1 and 1.6.2 illustrate the percent of wilted plants relative to days after chemical application. The tomato test crops showed greater effects from the analogue application than the pumpkins. The type of analogue used in the treatment had a pronounced effect on the tomatoes. Tomatoes treated with 8' acetylene ABA maintained their turgidity longer than the 8' methylene analogue. This difference was not apparent in the pumpkin trial.

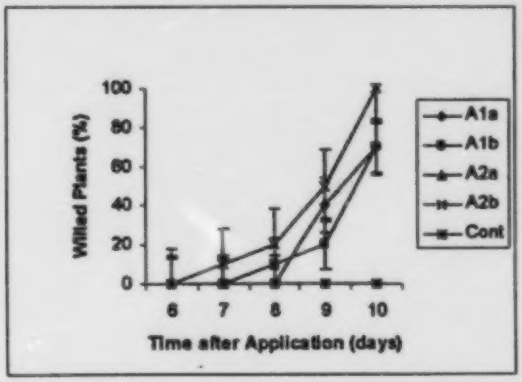


Figure 1.6.1. Percentage of tomatoes wilting as influenced by various ABA treatments.

A1 = 8' methylene
a = 5 X 10-5 M
A2 = 8' acetylene
b = 10-4 M

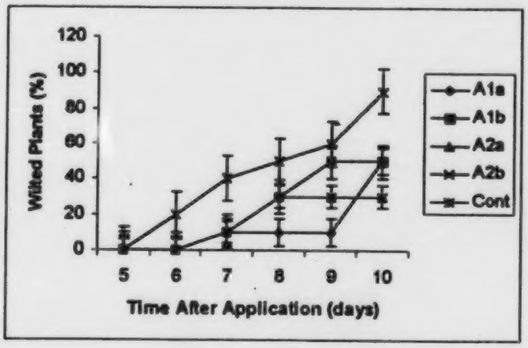


Figure 1.6.2. Percentage of pumpkins wilting as a function of different ABA treatments.

A1 = 8' methylene
a = 5 X 10-5 M

A2 = 8' acetylene
b = 10-4 M

Conclusion

These data confirm the previous findings;

a) that 8' methylene and 8' acetylene were effective in slowing water use and the onset of drought stress in tomato and pumpkin seedlings

b) that the effects of the ABA analog were dosage dependant and varied with the analog used.

Trial 1.7. Impact of Multiple Foliar Applications of ABA Analogs on Drought Stress Tolerance and Growth of Pumpkin Seedlings

Background. Previous trials (1.1 and 1.2) indicated that application of ABA analogs to pumpkins seedlings via either foliar sprays or root drenches had the potential to reduce water use - thereby potentially increasing the plants' ability to tolerate drought stress after transplanting. Root drenches provided long lasting effects but they required application of substantial quantities of the analogs. Foliar applications were equally effective as the root drenches but had a more limited duration of efficacy.

This trial was designed to determine the impact of multiple foliar applications of ABA analogs on drought stress tolerance of pumpkin seedlings.

Procedure:

Pumpkins (Curcurbita pepo cv. Jack o' Lantern) were seeded into Jiffy 7 on April 5, 1999 . The plants were grown out in the Department of Plant Sciences greenhouse (23°C/18°C, day/night), 14 h daylength with no supplemental lights. Once the plants reached the two true leaf stage (April 24), the plants were treated with the appropriate concentration of the ABA analogue PBI 365 or PBI 429 (provided by S. Abrams, PBI). Pumpkins are typically transplanted at about this developmental stage (2 weeks).

The treatments were 10^{-4} M PBI 365 or PBI 429 + Solvent as a foliar application;
one time : April 24
two times April 24 and May 2, 1999

Control = Water + Solvent applied as a foliar treatment.

The foliar application technique was outlined in Trial 1.2. An atomizer was used to apply the spray to leaf run off. The volume of spray required to treat one plant was about 9 mL. Tween at 0.1% was used as a spreader/sticker. Following the ABA treatments, the surface of each pot was covered with a 1 cm layer of perlite. The perlite eliminated evaporation as an avenue for moisture loss from the pots; thereby restricting moisture loss to transpiration. The plants were returned to the greenhouse and grown out under the previously described conditions. One group of plants for both the foliar and root dip treatments was watered as required over the next several weeks. This group was used to determine the impact of the ABA treatments under non-stressed conditions. The incidence of leaf yellowing was recorded in the non-stressed treatment, using a scale of 0= no yellowing to 5 = severe yellowing of all leaves. Water was withheld from the other group and the severity of wilting was recorded daily using a scale of 0 = no wilting to 5 = dead. There were seven replicates of each treatment and the treatments were arranged in a randomized complete block design of the greenhouse bench in order to correct for any gradients within the greenhouse. Each treatment replicate consisted of four plants. The second ABA treatment was applied at the onset of significant wilting. Following the second application of ABA, the plants were thoroughly rewatered then returned to the previously described conditions for a second round of observation. On May 16, (22 days after treatment) the stress trial was terminated as all plants had wilted. The non-stressed trial was terminated June 8 at which time the plants' growth characteristics (fresh weight and height of the tops, stem thickness, blossom number) were measured.

Results

Crop health was good in this trial.

Stressed Trial.

The seedlings were at a relatively advanced stage of development when treated. As a consequence water use was rapid and the plants wilted quickly. As indicated by Figure 1.7.1 and Table 1.7.1, the only treatment that consistently reduced wilting was PBI 429 applied twice. This treatment combined a high degree of efficacy with no significant phytotoxic effects.

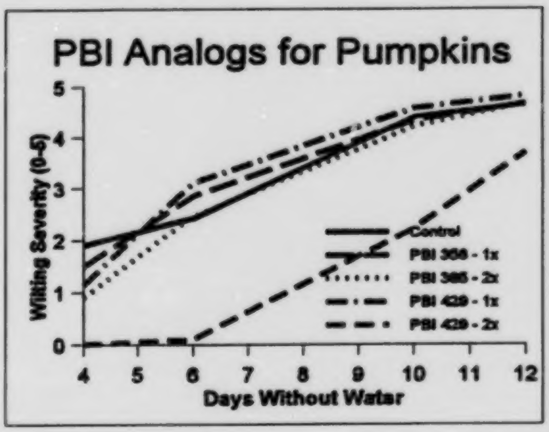


Figure 1.7.1 **Wilting severity data for pumpkins seedlings treated with ABA 365 or 429 either once or twice.**

Table. 1.7.1 **Wilting severity data for pumpkins seedlings with ABA 365 or 429 applied either once or twice as a foliar treatment.**

	Day 4	Day 7	Day10	Day 12
Control	1.92a	2.42a	4.39a	4.64a
PBI 365 x 1	1.50ab	2.85a	4.32a	4.67a
x 2	0.86b	2.46a	4.21a	4.67a
PBI 429 x 1	1.14ab	3.10a	4.57a	4.82a
x 2	0 c	0.10b	2.17b	3.71b
Contrasts				
ABA vs. Control	**	NS	*	NS
PBI 365 vs. 429	*	*	***	*
Single vs. Double Application	*	**	***	**

SEVERITY OF WILTING (0-5))=no wilting, 5 = completely wilted.

Non-stressed trial.

On average, plants treated with ABA were smaller (lower dry weight) than the corresponding controls (Table 1.7.2). Dry weights of the seedlings was not affected by either the analog applied or the number of times it was applied.

Table 1.7.2. Top dry weights for pumpkins seedling treated with foliar applied ABA analogs.

	Top Dry weight (g)
Control	0.65a*
PBI 365 - 1X	0.52b
PBI 365 - 2X	0.61ab
PBI 429 - 1X	0.58ab
PBI 429 - 2x	0.57ab

- Values within columns followed by the same letter are not significantly different (p=0.05)

Orthogonal Contrasts for Dry Weight data

ABA Analogs vs Control	.... **
PBI 365 vs PBI 429	.... NS
Single vs Double Application	.... NS

Conclusion

Multiple foliar applications of ABA 429 provided extended protection of pumpkin seedlings against drought stress. The ABA treatments slowed crop development. Some degree of retardation of crop development would be tolerated provided it did not lead to a longterm reduction in yields.

Section 2. Evaluation of ABA Analogues as "Holding" Agents for Potted Ornamentals.

Background

Trials in section 1 demonstrated that application of ABA analogues to transplants had the potential to slow water use and delay the onset of drought stress. The ABA treatments also slowed growth of the seedlings. A delay in growth may not be desirable in all cases, but in the nursery trade, the ability to "hold " plants at a particular stage is potentially very valuable.

Objective: ***To determine if ABA analogues can be used to temporarily slow growth and water use of potted ornamentals, thereby reducing maintenance and loss of quality during retail handling.***

Test Procedure

Petunias (*Petunia hybrida* cv Blue F1 Grandiflora) and marigold (*Tagetes patula* cv Sophia Mix) were selected for trial use as they are commonly grown bedding plants. Seeds were planted into Jiffy 7's at a rate of two seeds per pellet. These plants were grown in a greenhouse under day/night temperatures of 23°C/18°C with a 12 hour daylength period. A liquid fertilizer solution of NPK 20-20-20 (200 ppm) was applied weekly to meet plant nutritional requirements. At the two week growth stage, the plants were thinned to one plant per peat pellet.

After four weeks, the plants had reached a marketable size and were therefore considered to be ready for treatment with the ABA analogues. The plants were treated with 8' methylene, 8' acetylene, or a control solution. Both ABA analogues were applied at 10^{-4} M and 5 X 10^{-5} M concentrations. Each treatment contained ten plants. All chemicals were applied as root dips. The plants were placed into a solution containing the ABA and were allowed to soak for three minutes. Volumes of solution absorbed were recorded. The amount of chemical absorbed in the petunias ranged from 5 to 8 ml per plant, with an average of 6.2 ml. The average absorption per plant for marigold was 7.8 ml.

After chemical application, the plants were returned to the greenhouse. Plant water use was measured on a daily basis by weighing the plants in the peat pellets and then subtracting the weight from a known saturated peat pellet weight. Once these measurements were taken, the growth media was re-watered to field capacity.

Plant height measurements were recorded every third day. The evaluation period continued for ten days after chemical application. The plants were harvested after ten days, at which time fresh weight, height and degree of leaf chlorosis were measured. Chlorosis was evaluated on a scale of 0 to 5 with 0 representing no chlorotic symptoms and 5 representing extreme chlorosis.

Results

The ABA analogues treatments slowed growth in the both ornamental transplants as illustrated in Figure 2.1.1 and 2.1.2. Of the two crops tested, more pronounced effects were recorded in the petunias, however no significant differences were shown between the analogue types and concentrations in this crop. The type of analogue showed significant difference in the marigold crop, while the differences between analogue concentration were insignificant. In the petunias, the higher concentrations of both analogue treatments induced the onset of chlorosis. Chlorotic symptoms were not evident in the lower concentrations of the 8' methylene analogue treatment, and slight chlorosis occurred in the 8' acetylene treatment (Figure 2.1.3).

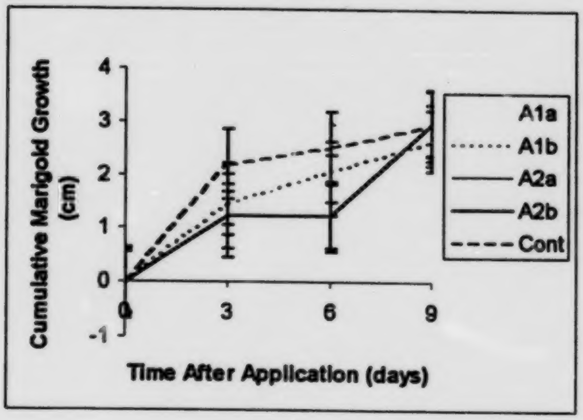


Figure 2.1.1. Growth in petunias after ABA analogue application.
A1 = 8' methylene
a = 5 X 10-5 M
A2 = 8' acetylene
b = 10-4 M

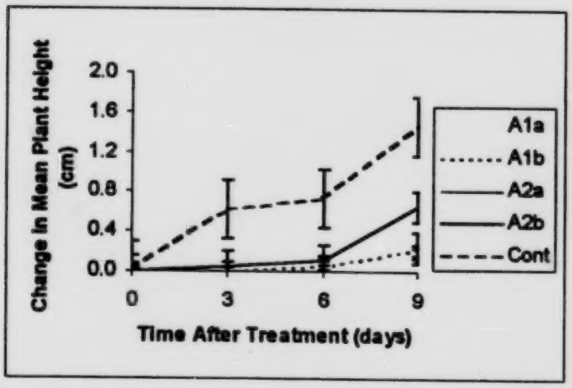


Figure 2.1.2. Growth of marigold after ABA analogue application.
A1 = 8' methylene
a = 5 X 10-5 M
A2 = 8' acetylene
b = 10-4 M

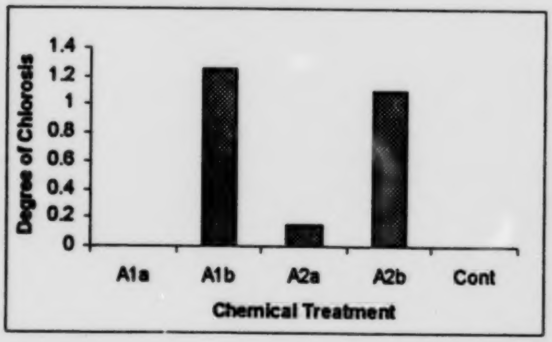


Figure 2.1.3. Degree of chlorosis onset in petunias treated with ABA analogues

A1 = 8' methylene
a = 5 X 10-5 M

A2 = 8' acetylene
b = 10-4 M

The water use for petunias and marigolds are represented in Figures 2.1.4 and 2.1.5, respectively. Moisture use (measured in ml of water per plant per day) was reduced by the ABA treatments in both test crop trials. In the cases of marigolds and petunias, the control treatments had almost consistently higher water use than the ABA analogue treatments.

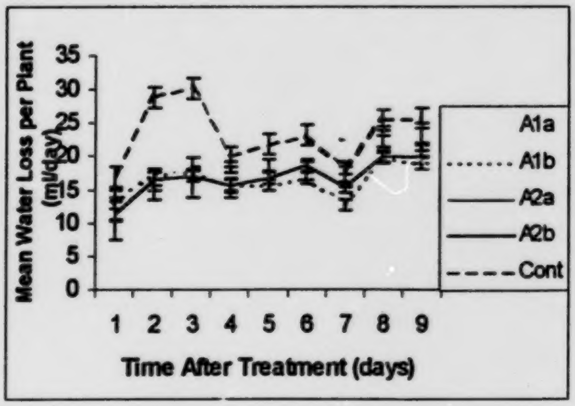


Figure 2.1.4. Daily moisture use in petunias treated with ABA analogues.

A1 = 8' methylene
a = 5 X 10-5 M

A2 = 8' acetylene
b = 10-4 M

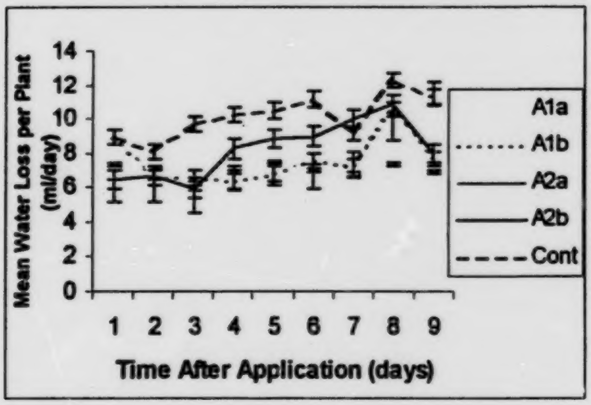


Figure 2.1.5. Daily moisture use in marigolds treated with ABA analogues.

A1 = 8' methylene
a = 5 X 10-5 M

A2 = 8' acetylene
b = 10-4 M

Plant leaf number and fresh weight measurements at the final harvest summarized in Figures 2.1.6 and 2.1.7, respectively. Control treatments had greater fresh weight and leaf number than the ABA chemical trials in both test crops. A more significant difference was observed in the petunia trial.

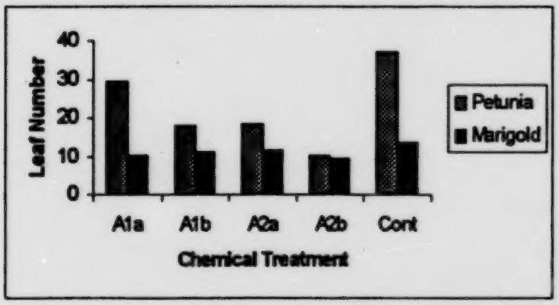


Figure 2.1.6. Influence of ABA analogues on leaf number in petunia and marigold trials at the final harvest.

A1 = 8' methylene
a = 5 X 10-5 M
A2 = 8' acetylene
b = 10-4 M

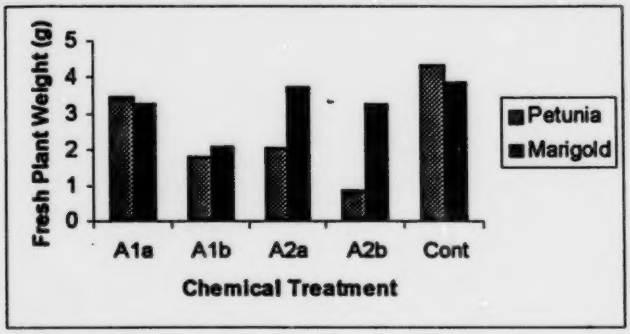


Figure 2.1.7. Influence of ABA analogues on fresh weight of petunia and marigold at the final harvest.

A1 = 8' methylene
a = 5 X 10-5 M
A2 = 8' acetylene
b = 10-4 M

Discussion

ABA naturally accumulates in plants during periods of stress. This accumulation triggers stomatal closure along with a slowing of cell growth. These changes, in turn, enable the plants to deal with drought, cold temperatures, and osmotic stress (Bray, 1993). The addition of exogenous ABA causes the plant to behave as it would under stress conditions. In this trial, ABA slowed growth and water use of the test crops.

From a retailer/producer perspective, these changes equate to decreased plant maintenance, reduced handling, and improvement in the overall quality of the bedding plants. The treatment related chlorosis observed in the petunia trail is undesirable. Further studies should be conducted to determine if the chlorosis observed in the petunias is permanent and could be avoided by additional nutrient supplements. Different crops should also be included as future considerations.

Trial 2.2 Use of ABA analogues as holding agents for horticultural crops.

Introduction

Abscisic acid is a well-known hormone, which helps control plant growth and stress physiology. ABA reduces water loss from the leaves by regulating the aperture of the stomata. In the longer term, ABA changes root growth to maintain water gathering activity under drought conditions. ABA- induced production of specialised and highly drought resistant roots in the plants of the Brassica family was reported by Vartanian et al., 1994 in Abrams S 1995). ABA induced protection against environmental stress involves retardation of plants' physiological processes like gaseous exchange and hence reduces photosynthesis. This reduction in metabolite production, in turn, slows plant growth by limiting cell division and elongation (Salisbury and Ross, 1992). Research on sunflower seedlings appear to indicate an inhibitory effect of root applied ABA on shoot and root growth (Lenzi et.al.1995 in Griffiths et.al. 1996). In contrast, ABA promoted the growth of excised Soybean roots (Yamaguchi and Street, 1977 in Griffiths et al., 1996). Abscisic acid applied to the rooting medium of intact, hydroponically-grown tomato (*Lycopersicon esculentum*) seedlings severely inhibited root growth at concentrations of 0.5 mmol m^{-3} (Griffiths et al., 1996). Increased ABA concentration in the apoplast surrounding the guard cells reduces stomatal conductance (Hartung and Davies, 1991).

Several plants growth retardant like paclobutrazol, uniconazol and ancymazol are commonly applied to container-grown ornamental and nursery plants to control stem elongation and produce compact plants thereby improving their appearance. Thus, growth-controlling chemicals may decrease plant maintenance and handling costs while improving overall quality and supply duration. However, for these products to be economically viable and commercially suitable the resulting retardation of growth should be reversible and non- phytotoxic.

In a previous greenhouse study (Section 2.1) in which two synthetic analogues of ABA were applied as root dip treatments to ornamental bedding plants (marigold and petunia), plant moisture use and growth were reduced significantly. Some degree of leaf chlorosis was observed in petunia plants treated with the higher concentrations of ABA. Similar results were observed in trials with the tomato and pumpkin conducted in the greenhouse under non- stressed conditions. Concentration, analog and crop specific phytotoxic effects were observed in most of cases. These studies suggested that ABA analogues might have potential as holding agents for ornamental and nursery raised plants provided effective in terms of dosages and mode of application are identified for specific crops.

This study was conducted to compare the effectiveness of two ABA analogues as holding agents, at similar doses and also to confirm the phytotoxic effects observed in the past studies.

Materials and Methods

Tomato (*Lycopersicon esculentum* cv. Manitoba) and marigold (*Tagetes petula* cv. Sparky) seedlings were raised in the Department of Plant Science greenhouse during the third week of January 2000. Seeds were planted into 85 cm^3 cell packs containing Sunshine Mix 4 (Sun Gro Horticulture Canada). Greenhouse conditions were $23^0C/18^0C$, day/night temperature and 14 hours daylength with supplemental light provided with halogen lamps $200wm^{-2}s^{-1}$. Five weeks old seedlings were treated with different concentrations (10^{-5} M, 5 X 10^{-5} M and 10^{-4} M) of 8' methylene (PBI 365) and 8' acetylene (PBI 429) solutions in acetone to evaluate the effects of ABA analogs as holding agents for nursery seedlings. The concentrations selected were based on the results from previous studies with ABA analogues conducted at the Department of Plant science Greenhouse. A solution containing water and 1% acetone was used as control treatment. Both analogues were applied as root dip treatments at identical concentrations for their comparison.

The seedlings were immersed in the appropriate concentration of ABA solution for two minutes. On average, the amount of ABA analog solution absorbed per seedling was 28 ml in tomato and 25 ml in marigold. Seedlings in cell packs were arranged in a Randomized Complete Block Design on a greenhouse bench. Each treatment was replicated four times and each treatment replicate consisted of six plants in the tomato and four plants in the marigold trial. Seedlings were fertilized with 200 ppm (20-20-20)-fertilizer solution every third day throughout the growth and evaluation period. The plants were watered once daily. The amount of daily water use by the seedlings was determined by weighing the plants in cell packs before watering and then subtracting that weight from the weight of saturated cell packs. Plant heights were measured every third day after treatment application.

The tomato trial was terminated ten days after treatment application. At that time, the control plants had grown too large relative to the moisture holding capacity of the cell packs and a single daily watering could no longer satisfy the transpiration demand of the control plants. The marigold trial was terminated twelve days after treatment application when there was no difference between control and treated plants in terms of daily water use.

At termination of the trial, the number of leaves per plant, fresh shoot weight and fresh root weight per plant were measured in tomato. In marigold, blossom number, fresh shoot weight and fresh root weight per plant were recorded.

All the plant growth parameters were analyzed using the GLM program of SAS. Repeated measure analyses were done for plant growth and daily water use over the period of evaluation. Where F values indicated significant (p=0.05) treatment responses, orthogonal contrasts (p=0.05) were used for comparison of the treatment effects while LSD tests (p=0.05) were used for comparison of treatment means. The following planned contrasts were made:

ABA treated vs Control
Analog type (PBI 365 vs PBI 429)
ABA linear (Concentrations)
Concentration quadratic
ABA*conc linear
ABA*conc quadratic

Results

Tomato

ABA analogues did not cause any visual phytotoxic effects in the tomato plants, nor did they imparted any significant developmental effect to the crop. The crop was absolutely healthy throughout the evaluation period.

Plant water use

ABA treatments significantly reduced the plant moisture use within two days of treatment application and continued to reduce the moisture use throughout the 10-day evaluation period (Figure 2.2.1). PBI 429 was

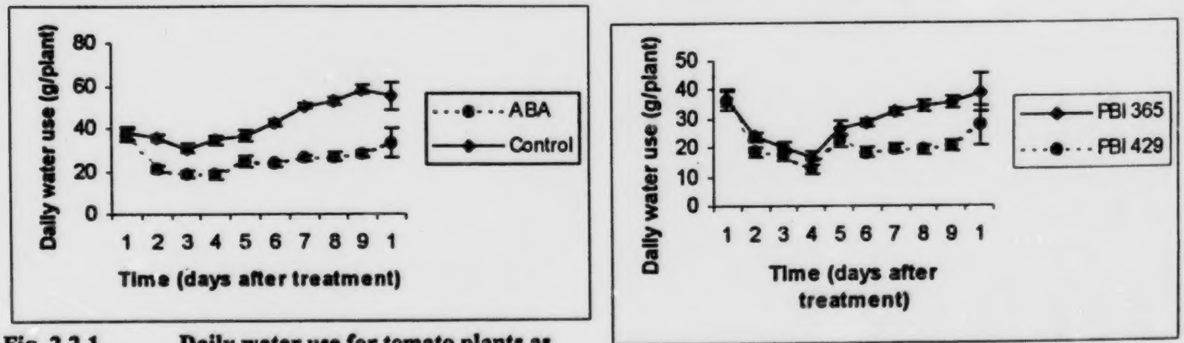


Fig. 2.2.1 **Daily water use for tomato plants as**

more effective in reducing plant moisture use than PBI 365 (Figure 2.2.1).

The concentration of ABA applied had a significant effect on the daily water requirement of the tomato seedlings. Daily water use showed a linear response to the concentration of chemical applied, with the highest concentration being most effective in reducing water loss by the seedlings. The ABA analog type and the interaction between analog type and concentration were highly significant from six days after treatment application until the ninth day.

The total amount of moisture used per plant during the evaluation period was significantly lowered by the ABA treatments (Figure 2.2.2). Total water use showed a significant linear and quadratic response to the concentration of ABA applied suggesting that water use declined with increasing ABA concentration in a curvilinear fashion (Figure 2.2.3). The analogue type had also significant effect on total water use of the tomato seedlings. The total water use by the PBI 429 treated plants was significantly lower than the plants treated with PBI 365 (Figure 2.2.2).

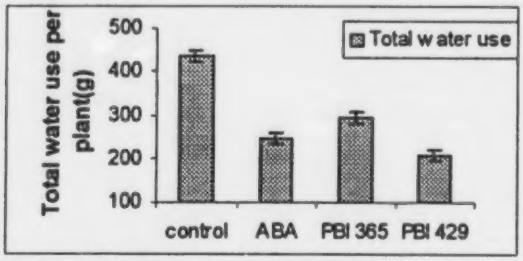


Fig 2.2.2 Total amount of moisture used by ABA analogue treated tomato seedlings throughout a ten-day evaluation period.

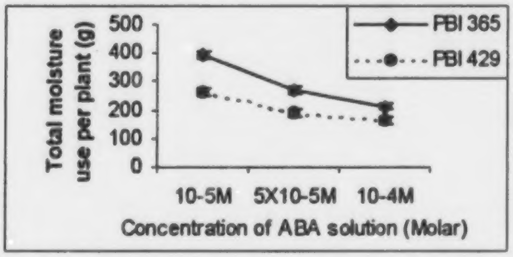


Fig 2.2.3. Total amount of moisture used by ABA analogue treated tomato seedlings throughout a ten-day evaluation period.

Plant growth

Cumulative plant growth (measured as a change in height from the beginning to the end of the evaluation period) was significantly reduced by the ABA treatments (Figure 2.2.4). Regarding the effect of analogue type, PBI 429 significantly reduced seedling growth compared to PBI 365(Figure 2.2.5). Similarly, the analogue type and concentration had significant effect on seedling growth (figure 2c). Seedling growth showed a curvilinear response to the concentration of chemical applied as suggested by the significant linear and quadratic components of the total treatment variation (Table 2.2.1). Similar results were reported in studies with Geranium plants root drenched with the plant growth retardants, paclobutrazol or uniconazole (Whipker et al., 2000).

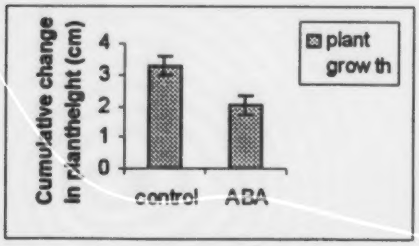


Figure 2.2.4. Influence of ABA analogs on seedling height.

Shoot fresh weight

Shoot fresh weight at the final harvest was significantly greater in control plants compared to those treated with ABA (Figure 2.2.6). Analogue type and the concentration applied had significant effects on shoot fresh weight. PBI 365-produced greater mean shoot fresh weights than PBI 429 (Figure 2.2.6). Similarly, shoot fresh weight showed a negative linear response to increasing concentrations of ABA. There was a significant interaction between analog type and concentration of chemical applied. PBI 429 treatments produced significantly lower mean shoot weights compared to the corresponding concentrations of PBI 365 (Figure 2.2.6).

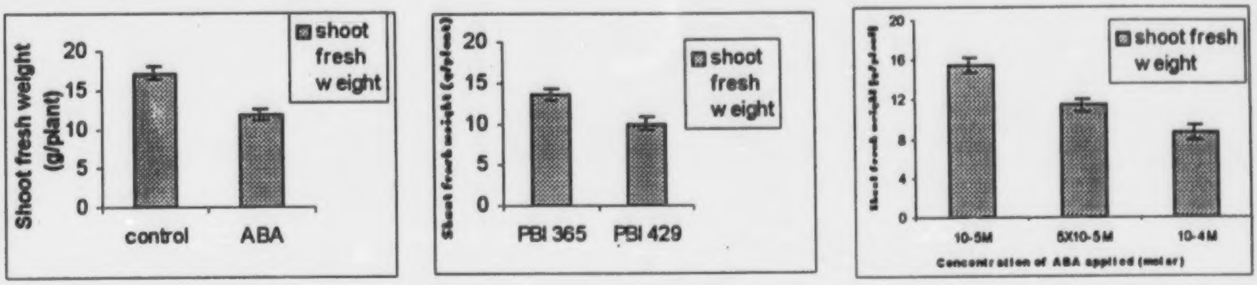
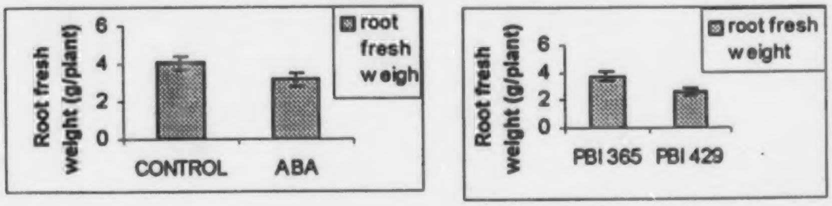


Figure 2.2.6. Influence of ABA analogs on shoot fresh weight of tomato seedlings.

Root fresh weight

The control treatments again had significantly greater mean fresh root weights compared to the plants given the ABA treatments (Figure 2.2.7). Analogue type and concentration of analogue applied also had significant effect on the root fresh weights. PBI 365-produced greater mean root fresh weights than PBI 429 (Figure 2.2.7). Similarly, fresh root weight showed negative linear response to the increasing concentrations of ABA applied. There was a significant interaction between analog type and concentration of chemical applied. PBI 365 treatments produced significantly greater mean root weights compared to the corresponding concentrations of PBI 429 (Figure 2.2.7).



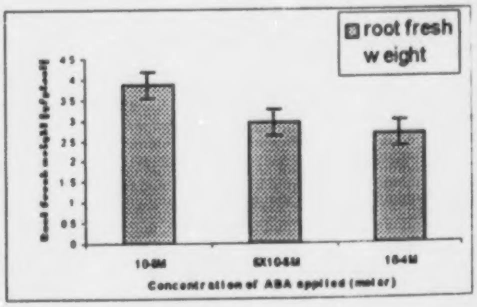


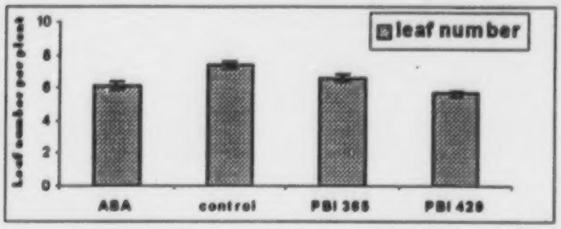
Figure 2.2.7. Influence of ABA analogs on root weights of tomato seedlings.

Root to shoot ratio

The concentration of ABA applied and the interaction between analog type and concentration used had significant effects on the root to shoot ratio of the tomato seedlings. However, the difference in mean root to shoot ratio between analog type and control and ABA treatment on average was insignificant. Mean root to shoot ratio showed a positive linear response to the increased concentration of the ABA applied. The effect of increased concentration of ABA was truly linear in PBI 429 but in PBI 365 the lowest and highest concentrations were statistically equivalent and significantly greater than the medium concentration (Table 2.1.1). The highest concentration of both analogues (10-4 M) and medium concentration of PBI 429 (5 X 10-5 M) recorded greater mean root to shoot ratio while other concentrations were statistically equivalent to the control (Table 2.2.1).

Number of leaves per plant

Leaf number per plant was significantly lowered by the ABA treatments (Figure 2.2.8). Similarly, the analog type and concentration of chemical used had significant effects on leaf number. PBI 429 treatments significantly reduced the number of leaves per plant relative to PBI 365. The number of leaves per plant was also reduced with increasing concentrations of ABA applied (Figure 2.2.8).



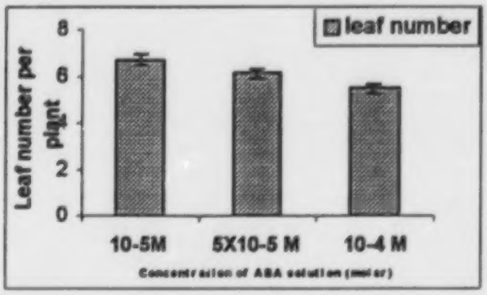


Figure 2.2.8. **Influence of ABA analogs on leaf number of tomato seedlings.**

Marigold

By contrast to the previous trials, no visual chlorosis and phytotoxic symptoms of ABA were observed in this trial. The crop was absolutely healthy throughout the evaluation period.

Plant water use

Total moisture use throughout the evaluation period was not significantly different between control and ABA treated marigolds. However, the type of analogue applied had a highly significant effect on total water use. PBI 429 treatments, on average, used significantly less water than the PBI 365 treatments (Figure 2.2.9).

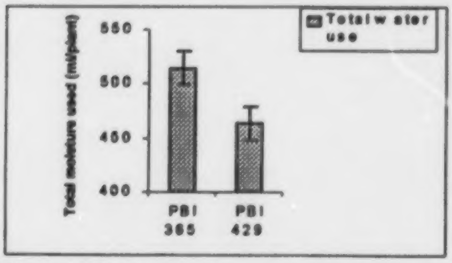


Figure 2.2.9. Total amount of moisture used by ABA analog treated marigold seedlings throughout a ten-day evaluation period.

Daily water use averaged over the twelve-day evaluation period was significantly different between the control and the ABA treated plants. Also there was a significant difference between the ABA analogues (Table 2.2.2). No significant interaction between treatments and concentration of the analog solution was observed. When the data from daily water use were analysed as simple RCBD model, a clear pattern of the effect of ABA analogues was observed. For the first three days, there was no significant treatment effect. The ABA treatments significantly reduced moisture use for the next four days and again there was no ABA effect on plant moisture use towards the end of the evaluation period (Figure 2.2.10). The PBI 429 treatments, on average, recorded lower mean moisture uses compared to PBI

365 when there was a significant difference between analogue types (Figure 2.2.10).

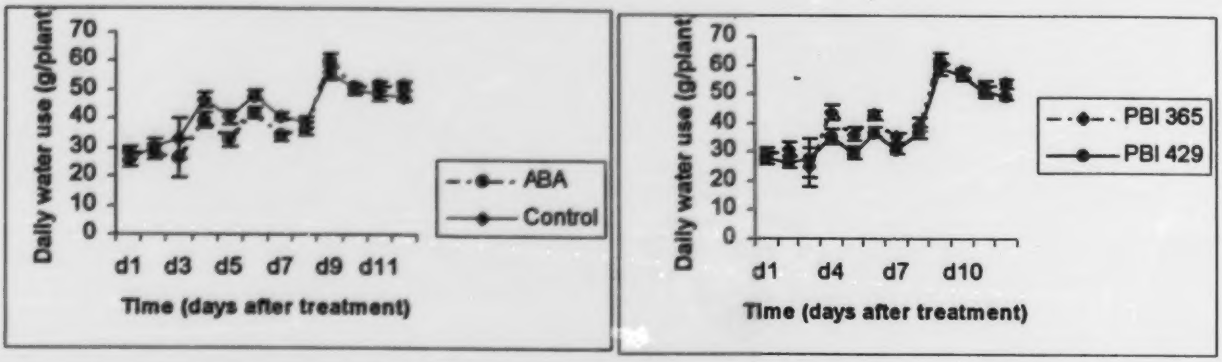


Figure 2.2.10. Daily water use by marigold seedlings as influence by aplication of two ABA analogs.

The ABA treatments had no obvious developmental effects compared to the control. However, the analog type showed significant effects on plant development. PBI 429 treatments had more fully developed flower buds compared to PBI 365 treatments at the end of the trial (Figure 2.2.11).

All other plant growth parameters (plant height, fresh shoot and root weight) were not influenced by the ABA treatments.

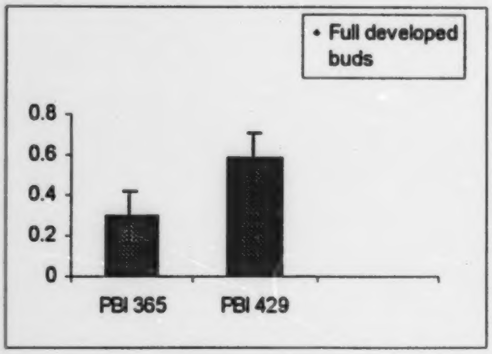


Figure 2.2.11. Effect of two different ABA analogs on flower bud number of marigold seedlings.

Discussion

In greenhouse studies previously conducted at the Department of Plant Sciences greenhouse at University of Saskatchewan, ABA treatments applied to plants under non-stressed conditions retarded growth and produced chlorosis depending upon analog type and concentration of chemicals used. The effects of ABA analogues were crop specific. The extent of chlorosis and growth retardation was relatively higher in PBI 429 treatments compared to PBI 365 and with higher concentrations (Section 2.1). ABA analogs had been demonstrated to control the moisture use and growth of marigold and petunia plants in one holding trial conducted in the greenhouse, but significant chlorosis was observed in petunia plants (Section 2.1).

In this trial, application of ABA as a root dip treatment did not cause yellowing as noted in previous study. However, the effects of the ABA analogues were again crop specific. The effects of ABA on plant growth were significant only in tomato. PBI 365 delayed plant development in marigold. On average, the PBI 429 treatments were more effective than PBI 365 treatments in preventing plant moisture loss in both test crops. The highest concentration of the chemical solution (10^{-4} M) was most effective for reducing moisture loss and slowing plant growth in tomato. The lowest concentration was relatively ineffective. In terms of total moisture used, the medium concentration was as effective as the highest concentration. PBI 429 as a root drench treatment was also observed as more efficient than PBI 365 in reducing plant growth and moisture use in previous studies with petunia and marigold and in non -stressed trials of greenhouse including tomato and pumpkin.

In the present study, the analog effects on plant growth and moisture use were as expected in tomato. The ABA analogues reduced moisture loss and retarded tomato plant growth based on concentration and analogue type used in the study. Similar results were reported in studies with Geranium plants root drenched with plant growth retardant, paclobutrazol or uniconazole (Whipker et al., 2000). Similarly, soil drenched paclobutrazol and uniconazole were reported to control plant height in ornamental cabbage and kale in one study conducted at the North Carolina State University (James et al. 2000). The ABA analogues as observed in a previous study conducted at the Department of plant science greenhouse did not significantly affect the plant growth in marigold. However, the reduction in moisture by the ABA treated plants was consistent over the range of crops used and across the years of study. Therefore, inconsistent and crop specific effects observed in these studies suggest further investigations with consideration of different crops. Furthermore, the retardation of plant growth only for an intended period of time with the possibility of rapid recovery afterwards is an important point to consider while selecting analogue type and appropriate concentrations.

The high moisture requirement of the nursery plants is one of the major maintenance costs incurred in handling, transportation and marketing. Nursery growers can potentially utilise these chemicals to check the moisture loss from the plants thereby ensuring efficient handling transportation and display for local and distant market without loss of visual quality. In crops, like tomato, where the ABA analogues can check both moisture loss and plant growth, these chemicals have an additional advantage of facilitating establishment of the transplants in field. Smaller, slower growing plants are less susceptible to physical damage at transplanting and require less moisture to avoid transplant stress. Before the ABA analogs can be recommended for commercial scale use, a comparative evaluation of cost effectiveness of the ABA analogs is necessary relative to the other plant growth retardants available to the nursery industry.

Conclusion

This study showed that ABA analogues applied as root dips to the nursery grown tomato seedlings can potentially reduce plant growth and water demand for a few days without causing any detrimental effects on visual quality. These treatment effects will allow effective handling, shipment, transplanting and display at the retail stores. Out of two analogues tested, PBI 429 showed consistently greater efficacy than PBI 365 and 10^{-4} M was the most effective and safe concentration identified in the crops tested so far.

Suggestions

Different nursery plants should also be considered as test crops to access crop specific effects of the ABA analogues and identify the suitability of analog application for a particular crop. As PBI 429 as root dip is consistently more effective than PBI 365 both in reducing water loss and controlling plant growth, we should consider only this analog (PBI 429) at different concentrations and in different test crops in future.

Table 2.2.1 **Treatment means for moisture use and growth characteristics of tomato seedlings treated with various concentrations of ABA analogues through root dip treatments after a ten day evaluation period.**

Treatments	TMU (g/plant)	DMU (g/plant)	Plant growth(cm)	FSW (g/plant)	FRW (g/plant)	RSR	Leaf #/plant
PBI 365							
1 x10^{-5} M	395.09	39.13	3.51	17.68	5.08	0.28	7.33
5 x 10^{-5} M	269.01	33.40	2.28	13.90	3.34	0.24	6.58
10 x10^{-5} M	213.56	28.11	1.39	9.40	2.75	0.29	5.92
PBI 429							
1 x10^{-5} M	265.33	33.53	2.51	13.40	2.68	0.20	6.13
5 x 10^{-5} M	191.73	25.70	1.10	8.98	2.56	0.29	5.71
10 x10^{-5} M	163.43	20.45	1.44	7.97	2.57	0.32	5.10
Control	434.57	43.12	3.31	17.26	4.06	0.24	7.42
LSD (p = 0.05)	38.79	8.11	0.90	2.18	0.95	0.05	0.68
Treatment SS	252647.11	4392.00	65.95	378.12	21.99	0.04	105
Contrast SS							
ABAvs.control	117183.28	1753.24	16.61	98.96	2.75	0.00	105.65
365 vs. 429	44090.93	875.29	9.17	75.33	7.47	0.00	34.32
ConcLin	77604.95	1714.36	28.81	184.14	5.71	0.02	34.02
ConcQd	7213.66	32.71	5.99	5.89	0.78	0.00	36.24
ABA*ConcLin	6166.86	11.31	3.63	9.02	14.71	0.01	0.04
ABA*ConcQd	387.60	5.79	1.72	4.77	0.53	0.00	0.79

Table 2.2.2 Treatment means for moisture use and growth characteristics of marigold seedlings treated with various concentrations of ABA analogues through root dip treatments after a twelve day evaluation period.

Treatments	TMU (g/plant)	DMU (g/plant)	FSW (g/plant)	FRW (g/plant)	Full devt. flower bud	Total# flower bud
PBI 365						
1×10^{-5} M	528.94	40.97	13.70	9.60	0.38	1.25
5×10^{-5} M	511.05	39.73	13.24	7.78	0.38	1.18
10×10^{-5} M	504.13	38.91	13.45	7.74	0.13	1.00
PBI 429						
1×10^{-5} M	479.92	37.69	12.67	7.81	0.63	1.31
5×10^{-5} M	463.42	34.98	13.01	7.12	0.56	1.19
10×10^{-5} M	449.10	36.78	13.62	7.14	0.56	1.38
Control	505.39	41.75	13.34	8.03	0.38	1.44
LSD (0.05p)	46.18	4.25	2.13	1.95	0.38	0.47
Treatment SS	19425.78	409.38	6.13	16.36	0.70	0.50
Contrast SS						
ABAvs.control	873.10	131.55	3.13	0.07	0.13	0.16
365 vs. 429	15337.37	206.35	0.45	6.45	0.51	0.127
ConcLin	3029.40	23.25	0.51	5.98	0.10	0.03
ConcQd	124.00	27.47	0.03	2.12	0.00	0.00
ABA*ConcLin	39.97	5.05	1.37	1.33	0.39	0.10
ABA*ConcQd	21.93	15.69	0.41	0.39	0.03	0.05

Section 3. Impact of ABA Analogs on Frost Tolerance of Horticulture Crop Seedlings.

Background

Trials in section 1 indicated that application of the ABA analogs slowed moisture use by seedlings of several horticultural crops. This should increase their tolerance of the drought stress commonly encountered at transplanting. Cold and frost are another form of stress commonly encountered by newly transplanted Horticultural crops. Natural ABA is known to increase cold stress tolerance in a transient manner. As the ABA analogues are known to have longer durations of efficacy that the natural form, they may prove useful in enhancing cold/frost tolerance.

Objective : *to evaluate the potential for increasing frost tolerance of seedlings of high value Horticultural species via application of ABA prior to the cold event*

Procedure:

Tomatoes (*Solanum esculenta* cv. Spitfire) and pumpkins (*Cucumis pepo* cv. Baby bear) were seeded into Jiffy 7 s. The plants were grown out in the Department of Plant Sciences greenhouse (23°C/18°C, day/night), 14 h daylength with no supplemental lights. On November 15 the plants were treated as a root dip with either PBI 365 or PBI 429 at 10^{-3}, 10^{-4}, 10^{-5} M. Water served as the control treatment. The plants were allowed to equilibrate for several days before exposure to the cold treatments. This equilibration time was design to allow for the ABA treatments to become fully effective, as some of the changes caused by treatment with ABA (changes in membrane structure and protein composition) take time to manifest.

The cold treatments were;
- a) 0 C for 4 hours
- b) -2.5 C for 4 hours
- c) - 5 C for 4 hours

Both pumpkins and tomatoes are renowned for their frost sensitivity. Non-acclimated plants typically show frost damage at -1C.

The cold treatments were imposed by moving the seedlings into temperature controlled growth chambers set at the appropriate temperature. All treatments took place in the dark.

Following the cold treatments, the plants were moved back to the greenhouse and maintained under standard conditions for several days during which time there responses to the cold treatments were observed.

Results

At the time of cold treatment there were no indications of the ABA affecting the growth or appearance of the seedlings.

None of the plants exposed to the 0 C conditions showed any symptoms of damage. Although both these crops are sensitive to chilling at temperatures below 5C, the duration of cold exposure in this trial was insufficient to cause chilling damage.

For both crops, all the plants exposed to the -2.5 or -5C treatments were extensively and permanently frost damaged irrespective of the analogue used or the concentration it was applied at. Symptoms of frost damage were apparent upon removal of the plants from the cold chambers (wilting and leakage of cell contents). These symptoms progressed to a generalized necrosis after several days in the greenhouse.

This trial suggests that the ABA analogues have limited potential to increase the frost tolerance of Horticulture crop seedlings - at least in the form and concentrations of ABA tested.

Trial 3.2. The Effects of ABA Analogs on Chilling Tolerance in Horticulture Crops.

Introduction

Chilling of plants occurs when the plants or tissues are exposed for extended periods to temperatures above 0 C but below a "damage threshold". This damage threshold depends on the crop and the tissue involved (typically 5-10 C). Chilling is responsible for large economic losses in horticulture crop production. Injury may occur at any stage of production from germination through to post-harvest (Wang, 1990). The climate in Saskatchewan represents a challenge for field production of horticulture commodities as it is often difficult to avoid chilling temperatures. Transplants are particularly vulnerable to chilling damage due to unpredictable environmental conditions in the spring.

The chilling tolerance of plants can be increased by utilizing production practices which slow growth and/or subject the plant to a limited amount of stress. Commonly employed methods to increase shilling tolerance include;

a) low temperature acclimation,
b) high temperature acclimation
c) reduced moisture availability
d) reduced nutrient availability

ABA is known to be involved in plant responses to stress. As ABA levels increase, there is an associated increase in stress tolerance including low temperatures stress.

Objective:

To determine the effective of ABA analogues on chilling tolerance in chilling sensitive horticulture crop transplants.

Materials and Methods

Pumpkins (*Cucurbita pepo* cv Jack 'O Lantern) and tomatoes (*Solanum esculenta* cv Roadside Red)were seeded into Jiffy 7's at a rate of two seeds per peat pellet. These plants were grown in the greenhouse with 23°C/18°C day/night temperatures with 12 hour day length. After one week of growth, the plants were thinned to one specimen per pellet. Fertilizer was applied on a weekly basis as a liquid solution of NPK 20-20-20. At the two week growth stage, the plants were treated with either 8' methylene or 8' acetylene ABA analogues at concentrations of either 10^{-4} M and 5 X 10^{-5} M. The ABA was applied as a root drench. Control plants were treated with water. The amount of solution absorbed by the pumpkins was 12.8 ml, while the average tomato absorption was 10 ml. After treatment, the plants were returned to the greenhouse for two days prior to undergoing chilling treatments.

The chilling regime involved placing the test crops into a 0°C growth chamber for 24 hours. A preliminary trial had been performed on untreated crop specimens to determine the length of exposure required to produce chilling damage. Trials of four, eight, ten, and twenty-four hours were reviewed. The 24 hour period produced substantial damage without killing the plants. Therefore the length of exposure was established to be 24 hours.

Wang (1990) states that chilling injury symptoms can appear soon after chilling or there may be a substantial time delay. The plants were evaluated immediately following the chilling procedure. Symptoms of chilling evaluated included leaf discoloration and wilting. Severity of chilling was rated on a scale from 0 to 5; with 0 representing no damage and 5 representing complete damage. After this initial evaluation, the plants were returned to the greenhouse to allow for development of any additional symptoms of chilling injury (Wang, 1990). These plants were grown for an additional week and chilling injury was then evaluated as previously described.

Results

The application of ABA analogues caused varied effects on chilling resistence in the test crops. In the tomatoes, initial chilling damage appeared to be reduced by the low concentrations of both 8' methylene and 8' acetylene ABA analogues (Figure 3.2.1). However, the chilling injury recorded after one week, was increased by the chemical treatments.

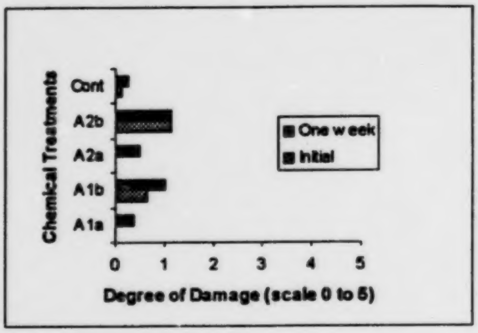


Figure 3.2.1. Initial and residual chilling damage of tomato (*S. esculenta* cv Roadside Red) after exposure to O°C for 24 hours.

A1 = 8' methylene a = 5 X 10-5 M
A2 = 8' acetylene b = 10-4 M

Figure 3.2.2 illustrates the results of the pumpkin trial. The 8' acetylene treatments showed greater chilling damage than the control in both initial and long-term measurements. The methylene analogue treatments, on the other hand, substantially decreased chilling injury.

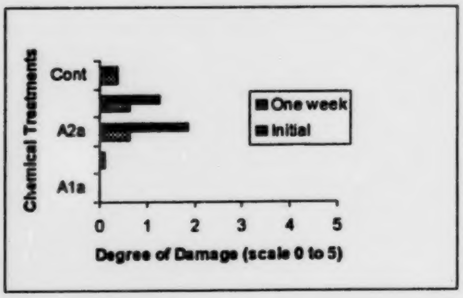


Figure 3.2.2. Initial and residual chilling damage of pumpkin (*C. pepo* cv Jack 'O Lantern) after exposure to 0C for 24 hours.

A1 = 8' methylene a = 5 X 10-5 M
A2 = 8' acetylene b = 10-4 M

Discussion

This preliminary trial suggests than application of ABA as a root dip may increase the chilling tolerance of horticultural crop transplants. The responses appear dosage dependant and analog specific. In certain situations, the analogs actually appeared to increase chilling damage. This effect should be investigated further. It is also possible that symptoms of ABA toxicity (leaf yellowing) were mis-diagnosed as chilling injury.

This trial will be repeated with the following modifications;

a) a non-chilled control will be used.

b) quantitative measurements of chilling injury (electrolyte leakage) will be employed in addition to the qualitative measures used previously.

Trial 3.3 Use of ABA analogues for protection of horticultural crops from chilling damage.

Introduction

Drought stress and cold shock are potential causes of transplant mortality during establishment of horticultural crops including vegetables and ornamentals. Both types of stress cause accelerated water loss from the plant resulting in tissue desiccation. Chilling injury occurs when plants or tissues are exposed to temperatures below a damage threshold for an extended period of time. The severity of chilling injury varies with the crop and tissue involved (Wang, 1990). Field conditions during the growing season in Saskatchewan are frequently challenging for production of horticultural commodities with chilling damage and drought stress, common problems. Crops of tropical origin such as pumpkin and tomato are particularly sensitive to low temperature injury during spring following transplanting in the field.

Abscisic Acid (ABA) is a hormone that mediates plant responses to environmental stresses such as cold drought and salinity. ABA's role in plants' drought and chilling avoidance mechanisms involves reducing transpirational water loss through stomatal closure. Exposure of plant tissues to temperature extremes results in changes in cellular membrane permeability which, in turn, results in leakage of water and solutes into the intercellular spaces (Wright and Simon, 1973). Water in the intercellular spaces is then subject to transpirational loss through the stomata. ABA induces stomatal closure, thereby reducing desiccation through prevention of water loss. Increased freezing tolerance, low temperature growth and advanced flowering was observed in ABA treated *Brassica napus* and *Brassica compestris* seedlings (Wilen et al. 1994).

Preliminary trials conducted with pumpkin and tomato, in the greenhouse suggested that application of ABA analogues as a root dip may increase the chilling tolerance of the transplants (Section 3.2). However, the effects of ABA were inconsistent and varied with the crop, analogue type and concentration. ***This study was conducted to clarify the previous findings in an effort to confirm the potential of using ABA analogues as chilling protectants in horticultural crops.***

Materials and Methods

Pumpkin (*Cucurbita pepo* cv. Jack O' Lantern) seedlings were raised in the Department of Plant Science greenhouse during the third week of January 2000. Seeds were planted into Jiffy 7 peat pots. Greenhouse conditions were 23°C/18°C, day/night temperature and 14 hours daylength with supplemental light. Four weeks old seedlings were treated with different concentrations (10^{-5} M, 5 X 10^{-5} M and 10^{-4} M) of 8' methylene (PBI 365) or 8' acetylene (PBI 429) solution in acetone to evaluate the effects of these ABA analogs on chilling tolerance.

A solution containing water and 1% acetone was used as the control treatment. Both analogues were applied as root dip treatments. The seedlings were immersed in the appropriate concentration of ABA solution for two minutes. On average, the amount of ABA analog solution absorbed per seedling was 24 ml. Treatments were arranged in a Randomized Complete Block Design on a greenhouse bench and each treatment was replicated four times with each replicate consisting of eight plants. The plants were fertilized with 200 ppm (20-20-20)-fertilizer solution every third day and were regularly watered daily throughout the evaluation period.

Two days after treatment application, four plants from each treatment were subjected to chilling treatment by placing them in a 0 °C- growth chamber for 24 hours. According to Wang (1990), chilling injury symptoms can appear during chilling and also after exposure to warmer temperatures. Therefore, immediately after the chilling procedure was completed, the plants were returned to the greenhouse and comparative chilling damage was recorded by measuring leaf chlorosis and tissue electrolyte leakage from both chilled and non-chilled plants. The plants were held in the greenhouse for an additional week and chilling injury and chlorosis were again evaluated. Leaf chlorophyll content was measured from newly developed second leaf, using a hand held chlorophyll meter (SPAD meter Model Minolta 502). Tissue electrolyte leakage was measured by taking ten 10 mm discs from newly developed second leaf from each treatment. The discs were immersed in 50 ml-distilled water and run in an electric shaker (190-rpm) at room temperature (21 oC) for seven hours. The electric conductivity of the solution was measured by using an Electrical Conductivity Meter (Model Orion 120) and the solution was then brought to boil in a microwave for about twenty minutes and cooled again to room temperature and total electric conductivity was measured. Relative electrolyte leakage of the leaf discs was calculated as the percentage of total electrolyte loss.

Data on leaf chlorophyll content and electric conductivity were analyzed using the GLM program of SAS. RCBD factorial model was used for analysis with ABA treatments as factor A and chilling treatment as factor B. Where F values indicated significant (p=0.05) treatment responses, orthogonal contrasts (p=0.05) were used for comparison of the treatment effects. LSD tests (p=0.05) were also used for comparison of treatment means. The following planned contrasts were made:

ABA treated vs Control
Analog type (PBI 365 vs PBI 429)
ABA linear (Concentrations)
Concentration quadratic
ABA*conc linear
ABA*conc quadratic

Results

The pumpkin seedlings developed a significant degree of chlorosis following treatment with ABA analogues (Figure 3.3.1). The analogue type and the concentration of the ABA applied had a significant effect on leaf chlorophyll content (Figure 3.3.2). PBI 429 treatments produced a greater degree of initial and residual chlorosis compared to PBI 365. Chlorosis was severest in plants treated with higher concentration of the

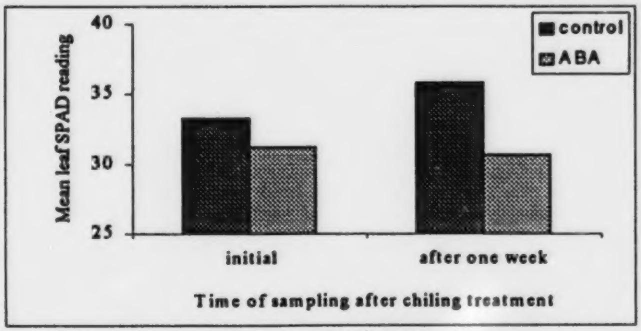


Fig. 3.3.1. Mean SPAD values for pumpkin leaves treated with ABA analogs.

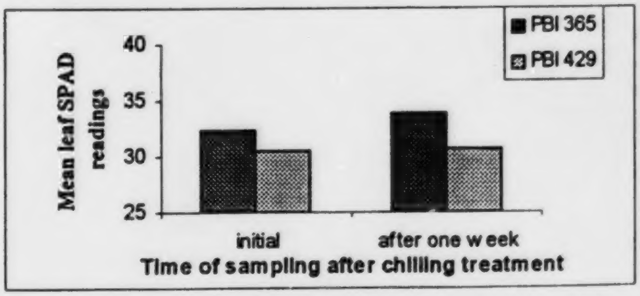


Figure 3.3.2: Mean leaf SPAD readings of pumpkin seedlings receiving different concentrations of two ABA analogues (PBI 365 and PBI 429) as root dip two days before chilling exposure.

analog solution (Table 3.3.1). The interaction between chilling and ABA treatment was not significant and chilling did not produce significant chlorosis compared to non chilled plants.

Electrolyte leakage as indicated by the relative conductivity of the chilled leaf tissue solution was not significantly influenced by the ABA treatments. Only the concentration of the ABA applied had a significant effect on the electrolyte leakage. The lowest concentration of PBI 365 increased electrolyte leakage compared to rest of the treatments including control (Table 3.3.1). The mean electric conductivity of the other ABA treatments was statistically equivalent to the control. Electric conductivity of the chilled plants was obviously greater than non- chilled controls. One week after chilling exposure, a significant difference in electrolyte leakage was observed between the chilled and non-chilled treatments but no ABA effects were evident (Table 3.3.1).

Table 3.3.1: Treatment means for leaf SPAD readings and relative electrolyte leakage of the Pumpkin seedlings treated with different concentrations of two ABA analogs as a root dip.

Treatments	Leaf chlorophyll content (immediately after chilling)	Leaf chlorophyll content (one week after chilling)	% Electrolyte leakage (immediately after chilling)	% Electrolyte leakage (one week after chilling)
PBI 365				
10 -5 M	34.57	35.13	39.87	40.00
5 x10 -5 M	32.43	35.53	33.37	39.75
10 x 10 -5 M	30.12	30.95	32.87	40.10
PBI 429				
10 -5 M	31.40	35.86	35.00	40.50
5 x10 -5 M	30.68	29.98	32.00	42.87
10 x 10 -5 M	29.27	26.16	33.00	39.25
Control	33.22	35.86	34.87	42.37
LSD (0.05p)	2.13	3.71	4.50	6.5

Discussion

In previous greenhouse studies, no significant chilling protection was observed in ABA treated pumpkin and tomato seedlings (Section 3.2). In this trial, the control plants and the plants given the lowest concentration of both analogues showed chilling damage soon after chilling. Application of higher concentration of ABA reduced chilling symptoms as indicated by mean electrolyte leakage. Chilling damage indicated by relative tissue electrolyte leakage was severe in lowest concentration of the PBI 365. One week after the chilling treatment, most of the plants had recovered except for the controls and those treated with PBI 365 at the lowest concentration. In previous trials, chilling injury was observed to increase for one week after chilling treatment. Since the lowest concentrations of both analogues were relatively ineffective, the over all effect of ABA was not obvious in this study. As the plants were returned to the greenhouse after chilling treatment and provided with appropriate temperature and moisture regimes for growth, most of the plants recovered from the temporary chilling. Similar recovery might not be expected under the less favorable conditions typically encountered in field situations. The control plants also recovered from chilling injury in this trial. Therefore, the duration/severity and efficacy of the chilling treatments used in this study is suspect.

Root application of ABA analogues produced severe chlorosis in this trial. Higher concentrations caused more chlorosis but the higher concentrations also helped reduce chilling damage.. In a study, where intact *Phaseolus vulgaris* seedlings were subjected to chilling (4 ^{0}C) in water saturated atmosphere, plants did not suffer water stress and ABA levels didn't increase over a period of 48 hours. However, when chilling lasted for 72 hours, a significant increase in ABA levels was found also in absence of water deficit (Vernieri et al., 1991).

Conclusion

Results observed in the present study were more or less similar to previous ones. The potential to use ABA analogues tpo protect plants against chilling damage is still unclear and needs further investigation. More appropriate concentrations and better chilling regimes need to be identified. Different crops (more or less chilling sensitive) should also be tested.

Section 4. ABA ANALOG FIELD TRIALS

Background :

Transplants of horticultural crops are very sensitive to soil moisture stress during field establishment. Attainment of normal water status through rapid root development in recently planted seedlings is essential for their establishment. Transplanting shock occurs in seedlings if the transpirational demand of a seedling shoot system exceeds the water uptake capacity of the root system. Thus, drought stress is a potent factor causing transplant mortality during establishment of horticultural crops.

Abscisic Acid (ABA) is a natural plant hormone involved in plant acclimatization to environmental stresses. ABA is found in all higher plants and has profound effects on a range of phenomena throughout the plants' growth and development. ABA seems to coordinate overall physiology of plants either by suppressing or promoting processes under varied circumstances (Addicott, 1983). ABA acts as a signaling molecule in various physiological processes including responses to environmental stresses such as drought, cold, salinity and heat (Abrams, 1999). ABA governs plants' drought avoidance mechanism that reduces transpirational water loss through stomatal closure. Under stress conditions, levels of ABA rise in plants. This triggers stomatal closure. As water potential drops in the root zone, levels of ABA in the root increase. This ABA is transported to leaves thereby inducing increased levels of ABA in the leaf which triggers stomatal closure (Hopkins, 1999). During drought stress, increased level of ABA in plants also inhibits solute uptake resulting in a drop in osmotic potential of the guard cells. However, the osmotic potential of subsidiary cells is hardly affected, so that a difference in turgor pressure between guard cells and subsidiary cells results in closure of stomata. ABA also regulates the expression of drought induced genes (lea type) involved in plants' adaptation to environmental stresses (Bray, 1997). Molecular studies with plants like Arabidopsis, tomatoes, tobacco, peas, *Zea mays* and wheat resulted in characterization of a large number of genes expressed upon dehydration of the plants in response to drought (Bartels *et al*, 1996).

Levels of ABA in plants can be increased through exogenous application of ABA. This ABA treatment can protect crops from desiccation by reducing transpirational water loss during drought stresses (Abrams, 1999). ABA applied to the epidermal strips of leaves triggers the leakage of the osmolyte malate from the epidermal strips which in turn, causes imbalances in K ion accumulation in the guard cells leading to loss of turgidity and hence inhibition of stomatal opening (Addicott, 1983). Exogenous application of ABA significantly increased the leaf resistance and water potential of pepper seedlings thus reducing transplant shock in greenhouse and field experiments (Berkowitz and Rabin, 1989). Similarly application of ABA to the cut ends of leaf petioles of *Phaseolus vulgaris* and sugarbeet caused rapid stomatal closure within eight to nine minutes (Kriedemann, 1972). Stomatal water loss at transplanting was significantly lowered in bare rooted Brussels sprouts seedlings treated with ABA (Mc-Kee, 1978). Increased freezing tolerance, low temperature growth and advanced flowering was observed in ABA treated *Brassica napus* and *Brassica campestris* seedlings (Wilen *et al.* 1994).

However, the protective effects of exogenously applied ABA are very short lived due, in part, to poor uptake and rapid turn over in the plant systems. To address this problem, chemically synthesized analogs of the hormone have been developed which can apparently mimic the effects of ABA. The alteration in the ABA structure causes the analogs to be metabolized more slowly than ABA, resulting in longer lasting and more effective biological activity (Abrams *et al.* 1997). Thus, ABA analogues can have great potential as crop protection chemicals, particularly for drought stress. The antitranspirant effects that the ABA analogues produced on barley leaves and water stressed cotton plants were found comparable with that of ABA

(Carbonmier *et al.* 1981). Grossnikel *et al.* (1996) reported improved survival of ABA analog treated spruce seedlings under conditions of water stress in the field. ABA analogues were found to increase chilling as well as freezing resistance in a number of crop plants including maize, wheat, rice, cotton, and cucumber. It appears that the ABA analogs trigger stomatal closure, which reduces water loss during chilling period (Dorffling *et al.*1989). Similarly, a number of ABA compounds applied as root drench to the rye seedlings enhanced freezing tolerance and improved root growth (Abrams, 1999).

Preliminary greenhouse researches on vegetables revealed that two ABA analogues namely PBI 365 and PBI 429 were effective in enhancing stress tolerance of a range of horticultural crops (Section 1 and 2). However, some dosage dependent phytotoxic effects were observed and the impact of the ABA analogues on rate of crop growth and yields were crop specific. In non-stressed trials, ABA treatment delayed plant growth and maturity and reduced yield as compared to the controls. Root drench treatments were more effective than the corresponding foliar application in terms of duration of efficacy. However, the root drench utilized more chemicals, so that multiple foliar application was considered to be more suitable from a cost-effective perspective. In a multiple foliar application trial, two applications of PBI 429 (10^{-4} M) was found promising, with a higher degree of efficacy and no significant phytotoxic effects. Foliar application of both ABA appeared to promote early flowering in tomato but root application did not produce the same effect.

Objective: ***a) To evaluate field performance of transplants of various horticultural crops treated with various rates of ABA analogues in advance of transplanting.***

b) to compare 8' acetylene ABA (PBI 429) with 8' methylene ABA (PBI 365) for efficcay and phytotoxicity.

Trial 4.1 1998 Field Trials

The trials utilized standard crop management practices. **These practices are designed to minimize all forms of stress on the crop throughout the production cycle.** Implementation of a "no-stress" environment allowed us to fully evaluate any negative effects of the ABA treatments that might arise if the product was used under optimal conditions.

The test crops were :

Pumpkin cv. Baby Bear

Tomato cv. Spitfire

Geranium - cv. Orbit Cardinal in 1998 and Impatiens cv . Regal in 1999.

Transplants of pumpkin and tomato were grown in the Plant Sciences Department greenhouse utilizing conditions and production practices previously described. The geraniums and Impatiens were purchased from a local nursery grower (Floral Acres, Saskatoon) as cuttings.

In the 1998 trial the treatments were;

Water + Solvent (Control)

$5x10^{-4}$, 10^{-4}, $5x10^{-5}$ 8' methylene (PBI 365) and 8' acetylene (PBI 429) ABA + Solvent

These rates were selected based on the responses obtained in Section 1. **Some of the higher rates had caused toxicity symptoms in the preliminary trials (see Section 1), but these rates were utilized in this trial as they had also been highly effective in protecting the transplants from stress.**

In 1998, all treatments were applied as a root dip to the transplants within three days of moving to the field. The root dip procedure employed is outlined in Section 1.1 of this report.

The pumpkins were two weeks old when treated, the tomatoes were five weeks old and the geraniums were eight weeks old and were at the 5th true leaf stage. The field trials were conducted at the Plant Sciences Department Horticulture Research Station in Saskatoon. This site is sheltered and features a Sutherland series clay soil. The soil was prepared prior to planting by discing and roto-tilling. Sufficient fertilizer was incorporated during the tillage to meet provincial recommendations for vegetable crops (100 kg N/ha, 120 kg P2O5).

The tomatoes and pumpkins were transplanted into wavelength selective mulch (IRT 76, 1.25 mil Market Farm Implements, PA) as soon as soil and air temperatures were warm enough to prevent chilling or frost damage. The pumpkins were covered immediately after transplanting with floating row cover (Agryl P-17). The crop covers accelerate growth and reduce crop moisture use. The covers were removed in mid-July at which time the plants had reached the limits of the tunnels and flowering was about to begin. In 1998, conditions following transplanting were extremely hot and windy - resulting in rapid dessication of the transplants irrespective of our efforts to protect the plants. These conditions resulted in significant mortality in the tomatoes. One week after transplanting, plant stands were evaluated and all dead plants replaced. Stand counts were taken again two weeks later. Conditions following transplanting were much more favorable in 1999 and 2000 and few plants were lost to transplant stress. Standard management procedures appropriate for each crop were employed for the remainder of the cropping season . Trickle irrigation lines installed beneath the mulch were used to maintain soil water potentials in the pumpkins and tomatoes above -30 kPa throughout the growing season. The in-row plant spacings were; 50 cm for the pumpkins and tomatoes with 3 m between rows. There were 10 plants in each treatment block.

In the 1998 trial, the geraniums were transplanted into a non-mulched area of the field on June 9. The geraniums were planted 0.3 m apart in rows 0.75 m apart with 10 plants/treatment row. In the 1999 trial the impatiens were transplanted in late June with 0.3 m between plants in rows 0.75 m apart with 10 plants/treatment row.

For all three crops, each treatment was replicated three or four times and the trial was arranged in a randomized compete block design.

Data collection

Stand counts were taken in all crops one and two weeks following transplanting.

The tomatoes were harvested weekly or biweekly at the onset of fruit ripening. Fruit harvest continued through until the first killing frost (September 28 in 1998, September 17 in 1999 and September 29 in 2000). Mature red fruit were counted and weighed at each harvest. Any offgrade-fruit were also harvested and weighed. Once frost killed the tomato plants, all remaining green fruit were harvested, counted and weighed.

The pumpkins were taken in a once over harvest in late September after the first killing frost. The fruit were counted and weighed and rated for extent of color change to orange.

In 1998, leaf and flower numbers were counted for the geraniums on several occasions over the course of the growing season. The relative vigor of the crop was also visually evaluated on several occasions. On September 18, the plants were harvested, the fresh weight of the plants was determined along with the total number of blooms per plant. In the 1999 trial, flower numbers were counted for the Impatiens on several occasions over the course of the growing season. The relative vigor of the crop was also visually evaluated on several occasions.

All yield and growth data were analysed using the GLM program of SAS (SAS Institute Inc., 1990). Where F values indicated significant (p=0.10) treatment responses, least significant difference tests (p=0.10) were used for comparison of treatment means. Single degree of freedom contrasts (p=0.10) were used to make broader treatment comparisons.

Results - Pumpkins (1998)

The results of the analysis of variance of the ABA treatment effects on pumpkins is presented in Table 4.1.1, while the treatment means and appropriate LSD values are presented in Table 4.1.2. Both ABA analogues reduced survival of the transplants relative to the control treatment, with PBI 429 having a greater negative effect on survival of the seedlings than PBI 365. As the rate of the analogue applied increased, survival decreased. The lowest concentration of PBI 365 produced survival rates equivalent to the control, while the highest rates of PBI 429 killed all but a few of the seedlings.

Table 4.1.1. F test Probabilities for Single Degree of Freedom Contrasts for Agronomic and Yield Parameters of Pumpkin Treated with Three Concentrations of Either 8' Acetylene (PBI 429) or 8' Methylene (PBI 365) ABA.

		FRUIT				
	Plant Stand	Number	Weight	Avg. Weight	Yield/ Plant	% Ripe
ABA vs Control	***	**	**	*	*	**
Analogue Type	***	**	**	**	0.67	**
ABA Concentration (Linear)	0	**	**	**	0.59	**
Analogue * Concentration (Linear)	0.43	0.23	0.18	0.99	0.22	0.68

Table 4.1.2. Treatment means for Agronomic and Yield Parameters of Pumpkin Treated with Three Concentrations of Either 8' Acetylene (PBI 429) or 8' Methylene (PBI 365) ABA.

		FRUIT				
	Stand (%)	#/Plot	Weight (kg/plot)	Avg. Wt. (kg)	Yield/Plant (kg)	% Mature
Control	96	152	133	0.87	14	42
PBI 429						
5×10^{-4}	6	13	9	0.68	27	2
10^{-4}	20	90	80	0.88	39	29
5×10^{-5}	3	21	18	0.85	26	6
PBI 365						
5×10^{-4}	13	53	44	0.83	42	27
10^{-4}	50	124	107	0.86	25	43
5×10^{-5}	76	137	117	0.86	15	24
LSD 0.05	*20*	*61*	*50*	*0.08*	*28*	*28*

Fruit numbers and weights were similarly affected by the ABA treatments (Table 4.1.2). On average, application of ABA to the seedlings resulted in a reduction in yields. This reduction in yields increased as the concentration of the ABA solution increased. PBI 429 had a more pronounced negative effect on yields than PBI 365. Yields for the lowest concentration of PBI 365 were statistically equivalent to the control. The negative impact of the ABA treatments on yields was largely due to their negative impact on the survival of the transplants. This is demonstrated by the fact that when yields were expressed on a per plant basis the surviving plants in the ABA treatments actually outyielded the controls. The increase in fruit/plant in the ABA treatments is probably more related to the increased space available for the surviving plants rather than any direct positive effects of the ABA on productivity.

The average fruit weight followed the same trends as outlined for the yields (Table 4.1.2). Application of ABA to the seedlings delayed maturity as indicated by the proportion of fruit showing a significant degree of de-greening prior to harvest. PBI 429 slowed fruit maturity more than PBI 365. For both ABA analogues, the 10-4 rate produced the highest proportion of fruit mature by harvest.

Tomatoes

The results of the analysis of variance of the ABA treatment effects on tomatoes is presented in Table 4.1.3, while the treatment means and appropriate LSD values are presented in Table 4.1.4. One week after transplanting, the ABA treated plants showed a significantly higher survival rate than the controls. However, many of the ABA plants showed symptoms of stress (leaf yellowing, localized necrosis) similar to those observed in the greenhouse trials. These symptoms could be attributed to the ABA treatments as; a) they were not apparent on the controls, b) the severity of the symptoms increased with the concentration of ABA applied and c) symptoms were much more severe for PBI 429 than PBI 365. When stand counts were taken two weeks after re-planting, the control and PBI 365 treatments had 100% survival whereas many of the newly replanted seedlings treated with PBI 429 had died. Mortality levels in this treatment increased with the concentration of analogue applied.

Table 4.1.3. F test Probabilities for Single Degree of Freedom Contrasts for Agronomic and Yield Parameters of Tomato Treated with Three Concentrations of Either 8' Acetylene (PBI 429) or 8' Methylene (PBI 365) ABA.

			MATURE FRUIT YIELD						
	Stand 1	**Stand 2**	**Sept9**	**Sept 18**	**Sept 26**	**Total**	**Green**	**Total**	**% Ripe**
ABA vs Control	*	*	***	***	0.12	**	0.39	**	***
Analogue Type	0.7	***	***	***	**	***	***	***	***
ABA Concentration (Linear)	0.92	*	0.6	0.41	0.93	0.44	**	**	0.79
Analogue Type * Concentration (Linear)	0.78	*	0.6	0.78	0.86	0.81	0.9	0.77	0.54

At the first two harvest dates (September 9 and 18), the control treatment produced more fruit than the pooled average of the ABA treatments. At these early harvests, the PBI 365 treated plants were higher yielding than PBI 429. The rate of ABA applied had no impact on yields at this time. By the 3rd harvest (September 26), yields for the PBI 365 treatments were equivalent to the controls, but PBI 429 yields still lagged behind. By the 3rd harvest, there was a consistent decrease in yields with increasing ABA concentration. At the conclusion of the trial the cumulative yields of ripe fruit for the control treatments was significantly higher than for the pooled ABA treatments. PBI 365 produced substantially more ripe fruit than PBI 429. The higher the concentration of ABA applied, the lower the final yields of ripe fruit. Plant given the highest concentration of PBI 429 did not produce any mature fruit by the conclusion of the trial. The yields for the lowest concentration of PBI 365 were somewhat higher than the control although the difference was not statistically significant at $p=0.05$. Total fruit yields (ripe+green) followed the same yield pattern described for the mature fruit. The data for percentage of ripe fruit indicates that the ABA treatments delayed maturity, with PBI 429 having a more pronounced effect than PBI 365.

Table 4.1.4. **Treatment means Agronomic and Yield Parameters of Tomato Treated with Three Concentrations of Either 8' Acetylene (PBI 429) or 8' Methylene (PBI 365) ABA.**

	Stand 1 (%)	Stand 2 (%)	MATURE FRUIT (kg/plot)						
			Sept. 9	Sept. 18	Sept. 26	Total	Green	Total	% Ripe
Control	56	100	5.3	11.5	5.6	22.3	100.1	122.4	18
PBI 429									
5 x 10^{-4}	66	56	0	0	0	0	21.5	21.5	0
10^{-4}	76	93	0.3	1.4	1	2.6	84.2	87.4	3
5 x 10^{-5}	93	96	0.5	5.2	2.6	8.3	105.5	115.2	7
PBI 365									
5 x 10^{-4}	63	100	0.5	4.3	3.5	8.3	95.3	104.8	8
10^{-4}	76	100	2.1	8.4	5.4	15.9	124.3	144	11
5 x 10^{-5}	86	100	4.3	13.8	7	25.1	106.8	133.9	19
LSD (0.05)	40	13	0.9	3.5	4.4	4.7	33.7	33	4

Geraniums

The results of the analysis of variance of the ABA treatment effects on geraniums is presented in Table 4.1.5, while the treatment means and appropriate LSD values are presented in Table 4.1.6. On June 18, two weeks after the crop was transplanted, stand counts were taken, and the surviving plants were evaluated for leaf number, bloom number and crop vigor (1 = poor, 5= excellent). Overall, the ABA treatments had no impact on stand relative to the controls. However, PBI 429 clearly reduced the stand relative to PBI 365. Leaf number was not significantly affected by the ABA treatments. However, flower number for the ABA treatments was significantly lower than the controls. This effect was effectively restricted to the plants receiving PBI 429. All plants showed symptoms of transplant stress at this time and vigor ratings were uniformly low. On July 17, the crop was again evaluated for vigor on a 1-5scale. At this time, the control treatments looked more vigorous on average than the plants treated with ABA. However, this effect was largely restricted to PBI 429; plants treated with PBI 365 were generally comparable to the controls. On August 20 the crop was again rated for vigor - this time using a scale from 7 = very poor through to 1= excellent. The response trends were similar to those observed on July 17. The highest rates of PBI 429 looked exceptionally poor by this point. On August 20 bloom counts were also taken, but variability between replicates obscured any treatment effects. At the final harvest (Sept 18), treatments receiving the highest levels of either analogue had lower plant fresh weights than the controls. Treatment with ABA analogues reduced the average number of blooms/plant at the final harvest. The higher the concentration of ABA applied, the greater the negative impact on final bloom count.

Table 4.1.5. F test Probabilities for Single Degree of Freedom Contrasts for Agronomic and Yield Parameters of Geraniums Treated with Three Concentrations of Either 8' Acetylene (PBI 429) or 8' Methylene (PBI 365) ABA.

	36694				36723	36757		Final	
	Stand	Leaf #	Bloom #	Vigor	Vigor	Vigor	Bloom #	Plant Wt. (kg)	Blooms /plant
ABA vs Control	0.18	0.47	***	0.71	***	**	0.51	0.17	**
Analogue Type	***	0.28	***	0.68	***	**	0.46	0.24	0.51
ABA Concentration (Linear)	0.12	0.56	***	0.47	**	*	0.27	0.19	***
Analogue * Concentration (Linear)	0.34	0.49	0.14	0.93	0.23	0.46	0.66	0.57	0.67

Table 4.1.6. Treatment means Agronomic and Yield Parameters of Geraniums Treated with Three Concentrations of Either 8' Acetylene (PBI 429) or 3' Methylene (PBI 365) ABA.

	36694				36723	36757		Final	
	Stand (%)	Leaf #	Bloom #	Vigor (1-5)	Vigor (1-5)	Vigor (1-7)	Bloom #	Plant Wt. (kg)	Blooms /plant
Control	100	73	122	2.3	4.6	1.7	114	0.61	22.9
PBI 429									
5×10^{-4}	63	47	9	0.08	1	7	57	0.36	13.7
10^{-4}	93	62	74	1.7	2.3	5.3	88	0.51	17.7
5×10^{-5}	96	57	80	1.7	2	4.2	92	0.58	18.5
PBI 365									
5×10^{-4}	93	57	87	1.5	3	4.5	83	0.44	17.8
10^{-4}	100	67	117	2.2	4	3.7	77	0.61	20.6
5×10^{-5}	100	76	118	2.2	4.3	1.4	114	0.58	22.8
LSD 0.05	15	31	17	1.5	0.7	1.3	60	0.18	5.2

In summary the impact of the ABA analogues on survival of transplants, rate of crop development and yields was crop specific. However, some general trends emerged;

a) the ABA treatments often had a negative effect on transplant survival. This seemed to attributed to direct toxicity of the ABA rather than to an increase in sensitivity to field stress. Any seedlings treated but not immediately planted also showed these symptoms. Similar symptoms of ABA toxicity had been observed in the preliminary screening trials in the greenhouse. These treatments were nonetheless considered potentially useful as they also had produced a dramatic increase in crop stress tolerance.

B) the ABA analogues slowed crop development resulting in reduced yields. Many of the seedlings which survived the ABA treatments showed sub-lethal symptoms of damage (leaf yellowing, stunting). This damaged slowed development of the seedlings thereby retarding crop development. By mid-season, all obvious symptoms of the ABA treatments had disappeared except that the treated plants were smaller and less developmentally advanced than the controls.

C) as the concentration of ABA applied was increased the intensity of any observed effects also increased.

D) PBI 429 generally had a greater effect than PBI 365. This was expected as PBI 429 had shown greater activity in preliminary assays. As most of the effects observed in this trial were negative, treatment with PBI 429 did not produce desirable results.

E) treatment with low concentrations (5x10-5) of PBI 365 produced growth and yield results comparable to and in some cases superior to the controls even in the absence of any significant crop stress.

F) none of the ABA treatments had any obvious impact on frost stress tolerance of the crops as indicated by the uniformity of damage observed following the September 28 frost.

It is noteworthy that every effort was made to minimize crop stress at planting and throughout the trial. It is therefore perhaps not surprising that the ABA treatments had little beneficial effect on survival or growth. ABA should be of maximum benefit in situations of crop stress (drought or cold). We did note that some of the ABA treatments did enhance survival of the tomato seedlings which were exposed to some dessication stress at transplanting. This suggests a potential role for the analogues as an insurance measure against adverse conditions.

Trial 4.2 1999 Field Trial of ABA analogs

This trial was designed to build on the results obtained from the 1998 field trial outlined in Section 4.1. plus the refinement in treatments observed in the greenhouse trials conducted over winter in 1998-1999.

MATERIALS AND METHODS

Transplants of pumpkin (*Cucurbita pepo* cv. Baby Bear) and tomato (*Lycopersicon esculentum* cv.

Spitfire) were raised in the Department of Plant Science greenhouse during the second week of May 1999. Pumpkin seeds were seeded into Jiffy 7 peat pots where as tomato seeds were seeded into 85 cm^3 cell packs containing Sunshine Mix 4. Greenhouse conditions were 23°C/18°C, day/night temperature and 14 hours daylength with no supplemental light. Three weeks old seedlings were treated with different concentrations of 8' methylene (PBI 365) and 8' acetylene (PBI 429) solutions in acetone two days before transplanting into the field to evaluate the effects of ABA analogs on field performance of transplants. A solution containing water and acetone was used as the control treatment. Both analogues were applied as foliar spray and root drench at identical concentrations for their comparison. On average, 10-ml solution of ABA was applied to each seedling as foliar spray and 90- ml in the root drench treatment of each seedling.

In the root dip treatments, the plants were immersed in the appropriate concentration of ABA solution for four minutes. In the foliar applied treatments, an atomizer was used to apply the spray to leaf run off. No spreader/ sticker was used.

The field trial management and data collection were conducted as outlined in Section 4.1.

RESULTS

4.2.1 PUMPKIN

The F values and treatment means from the single degree of freedom contrast of the ABA treatment effects on pumpkin growth and yield are presented in Table 4.2.1. The treatment means and LSD values for agronomic and yield parameters of pumpkin treated with different concentrations of the two ABA analogues either as a foliar spray or a root drench are presented in Table 4.2.2.

Table 4.2.1: F test probabilities and treatment means for single degree of freedom contrasts for agronomic and yield parameters of pumpkin treated with varying concentrations of either PBI 365 or PBI 429 as foliar spray or root drenches.

Trt. Contrast	% Stand	Yields(kg/plot)	% Orange fruit
Control vs. ABA			
Control	92	42	60
ABA	45	22	57
F value	***	***	0.1578
PBI 365 vs. 429			
PBI 365	66	32	67
PBI 429	24	11	46
F value	***	***	***
Foliar vs. root			
Foliar	74	33	71
Root	24	4	35
F value	***	***	***

Table 4.2.2 **Treatment means for agronomic and yield parameters of pumpkin treated with varying concentrations of either PBI 365 or PBI 429 as foliar spray or root drenches.**

Treatments	% Stand	Yield (kg/plot)	Avg. wt.(kg)	% Orange fruit
Control	92	42	0.76	59
PBI 365				
10^{-4} M Foliar	78	38	0.79	70
10^{-4} M Root drench	48	11	0.92	70
5×10^{-4} M Foliar	67	44	0.79	67
10^{-3} M Foliar	70	35	0.73	62
PBI 429				
10^{-4} M Foliar	69	28	0.79	73
10^{-4} M Root drench	0	0.0	0.00	0
5×10^{-4} M Foliar	17	17	0.74	0
10^{-3} M Foliar	8	.57	0.57	50
LSD (0.05)	21	16	0.18	16

Seedling survival

Application of the ABA analogues had a negative effect on seedling survival, with PBI 429 having a greater negative effect than PBI 365. Root drench treatments were more detrimental than the foliar application at the same concentration. The concentration of the ABA analogs applied had no significant effect on survival. The treatments that produced survival rates statistically equivalent to the controls were foliar application of PBI 365 at 10^{-4} M and 10^{-3} M and PBI 429 at 10^{-4} M (Table 4.2.2).

Fruit maturity

PBI 429 and root drench treatments delayed maturity relative to PBI 365 and foliar application respectively. Yields of ripe fruits for foliar applied PBI 365 were statistically equivalent to the control at all concentrations (Table 4.2.2).

Yields

Application of ABA to the seedlings resulted in a reduction in yields relative to the control. ABA 429 had a greater negative effect on yields than ABA 365. Foliar applied treatments with ABA 365 produced yields equivalent to the control (Table 4.2.2). The lowest yields were recorded for ABA 429 applied as root drench. Thus, foliar application was again found superior to the root drench.

4.2.3 TOMATO

The F values from the single degree of freedom contrast of the ABA treatment effects on tomato growth and yield are presented in Table 4.2.3. The treatment means and LSD values for agronomic and yield parameters of tomato treated with different concentrations of the two ABA analogues either as a foliar spray or a root drench are presented in Table 4.2.4.

Table 4.2.3: **F test probabilities and treatment means for single degree of freedom contrasts for agronomic and yield parameters of tomato treated with varying concentrations of either PBI 365 or PBI 429 as foliar spray or root drenches.**

Trt. Contrast	% Stand	Yields(kg/plot)	Avg. wt.(kg)	% Ripe
Control vs. ABA				
Control	100	**36**	0.08	**0.78**
ABA	99	**32**	0.09	**0.46**
F value	0.4597	0.6541	0.2195	0.932
PBI 365 vs. 429				
PBI 365	99	34	0.09	0.46
PBI 429	99	30	0.08	0.46
F values	1.00	0.1538	0.0852	0.100
ABA Linear				
10^{-4} foliar	100	**40**	0.10	**0.68**
5x 10^{-4} foliar	99	31	0.09	0.50
10^{-3} foliar	100	31	0.09	0.52
F value	0.1822	***	0.1097	***
ABA Linear				
10^{-4} foliar	100	40	0.10	0.68
5x 10^{-4} foliar	99	31	0.09	0.50
10^{-3} foliar	100	31	0.09	0.52
F value	0.1822	***	0.1097	***
Foliar vs. root				
Foliar	100	**40**	0.10	**0.68**
Root	99	**27**	0.09	**0.14**
F value	0.3415	0.9674	0.0545	0.8524

Table 4.2.4. **Treatment means for agronomic and yield parameters of tomato treated with varying concentrations of either PBI 365 or PBI 429 as foliar spray or root drenches.**

Treatments	% Stand	Yield (kg/plot)	Avg. wt.(kg)	% Market	% Ripe
Control Foliar	100	33.85	.10	55	0.78
Control Root drench	100	37.73	0.075	73	0.77
PBI 365					
10^{-4} M Foliar	100	39.94	0.11	61	0.17
10^{-4} M Root drench	97	29.13	0.09	58	0.28
5×10^{-4} M Foliar	100	34.21	0.09	69	0.87
10^{-3} M Foliar	100	33.60	0.99	62	0.53
PBI 429					
10^{-4} M Foliar	100	39.72	0.09	67	1.19
10^{-4} M Root drench	100	24.18	0.08	51	0
5×10^{-4} M Foliar	97	28.54	0.09	56	0.14
10^{-3} M Foliar	100	27.71	0.08	63	0.51
LSD (0.05)	4	8.74	0.03	18	1

Seedling survival

ABA treatments had no detrimental effects on seedling survival and plant establishment was uniformly high in all treatments including the control.

Fruit maturity

Fruit maturity was delayed with higher concentrations and the root drenches treatments of ABA. Foliar application of ABA 365 at the lowest concentration and ABA 429 at 10^{-4} M produced yields of ripe fruits statistically equivalent to the controls (Table 4.2.3).

Yields

There was an obvious effect of concentrations of ABA applied on the total yield of fruits, with highest concentration having a greater negative effect. Both the lowest and highest concentrations of ABA treatments recorded poor yields as compared to the medium concentration (10^{-4}). Some treatments like foliar applications of ABA 365 at all concentrations and 10^{-4} M of 429 produced yields equivalent to the controls (Table 4.2.4). Foliar application of both ABA analogues (PBI 365 and PBI 429) produced significantly higher yields than the corresponding root drench treatments. Marketable fruit yields were similarly affected by the ABA treatments.

DISCUSSION

In general, the ABA treatments had negative effects on establishment and the overall performance of pumpkin transplants. This corresponds with the findings of the trials conducted in 1998. Clear effects of analog type and mode of application of treatment i.e. foliar vs. root drench was observed this year. On the average, PBI 365 was superior (i.e. less negative) to PBI 429 while foliar application seemed less detrimental than the root drench treatment and also utilized less solution of ABA. More detrimental effects of root drench treatment may be due to higher amount of chemical involved in this method of application. Foliar application of both ABA's at 10^{-4} M was the most promising ABA treatment for pumpkin as it imparted less negative effect on seedling survival and over all performance.

In tomato, plant establishment was uniformly high in all treatments. ABA had no negative effect on seedling survival this year. PBI 429 had negatively effected the seedling survival in the 1998 trial (Section 4.1). Linear effects of ABA concentrations on yields and fruit maturity correspond to the last year results though the trend in concentration effects is not clear this year. Foliar application of both ABA types at 10^{-4} M seems to be superior to all the other concentrations. Higher concentrations caused detrimental effects on maturity and marketable yields. In general, performance of the plants foliar sprayed with PBI 365 were closer to the controls as compared to PBI 429. Foliar application of both ABA's at 10^{-4} M outperformed other treatments in terms of yields and maturity while root drench treatments at the same concentration performed poorly.

CONCLUSION

This study demonstrated that the application of ABA analogs to pumpkin and tomato seedlings prior to transplanting had no obvious positive effects on survival growth and yield potential in the absence of any significant stress. In the absence of crop stress, the ABA treatment often imparted negative effects like reduced survival, delayed maturity and reduced yields. However, performance of some ABA treatments were comparable with the control and even superior i.e. they produced higher yields than the control, though not significant at 0.05p level. Foliar application proved more effective than root drench treatment and utilized less chemical and labor. Thus, if appropriate treatment combinations are further fine-tuned, ABA analogues could be used to treat the seedlings as preventive measure against unpredictable weather situations so as to secure good plant establishment and yields. Due to their negative side effects, ABA treatments are not recommended for well-established growers capable of maintaining optimal growing situations for their crops.

Trial 4.3 Field Trials for 2000

When ABA analog treated pumpkin and tomato seedlings were grown under well-managed conditions of field (1998 and 1999 yield trials), the effects of ABA analogs on survival and overall yield performance were either negative or neutral. ABA analogs delayed the maturity and reduced yield in dosage dependent manner. Foliar applied PBI 365 was consistently superior to PBI 429 in most of the previous greenhouse studies (non-stressed sets) and in the 1999 field trials.

This study was conducted to confirm the previous findings observed with PBI 365 and identify a safe and effective concentration and timing of application.

Materials and methods

Tomato (*Lycopersicon esculentum* cv. Manitoba) and pumpkin (*Cucurbita pepo* cv. Baby Bear) seedlings were raised in the Department of Plant Science greenhouse during the first week of May 2000. Tomato seeds were planted into 85 cm^3 cell packs containing Sunshine Mix 4. Greenhouse conditions were 23^{0}C/18^{0}C, day/night temperature and 14 hours daylength with no supplemental light. Four weeks old seedlings were treated with three different concentrations of 8' methylene (PBI 365) solutions in 1% acetone two days before transplanting into the field to evaluate the effects of ABA analogs on field performance of transplants. Only the 365 analog was used in the 2000 field trial based on ht e results obtained in previous trials. The concentrations selected were based past two years of field trials results. A solution containing water and acetone was used as the control treatment. All the treatments were applied as a foliar spray. On average, 11-ml solution of ABA was applied to each tomato seedling with $$ ml applied to each pumpkin seedling. A summary of the treatments is presented below.

T1 – **PBI 365** 1 X 10-5 M two days before transplanting.
T2 - **PBI 365** 1 X 10-5 M two days BTP + 1 week ATP
T3 - **PBI 365** 1 X 10-5 M two days BTP + 1 week ATP + 2 weeks ATP
T4 – **PBI 365** 5 X 10-5 M two days before transplanting.
T5 - **PBI 365** 5 X 10-5 M two days BTP + 1 week ATP
T6 - **PBI 365** 5 X 10-5 M two days BTP + 1 week ATP + 2 weeks ATP
T7 – **PBI 365** 10 X 10-5 M two days before transplanting.
T8 - **PBI 365** 10 X 10-5 M two days BTP + 1 week ATP
T9 - **PBI 365** 10 X 10-5 M two days BTP + 1 week ATP + 2 weeks ATP
T10- **Control**

An atomizer was used to apply the spray to leaf run off. No spreader/ sticker was used.

The field management and data collection procedures utilized in the 2000 field trial were comparable to those outlined in Section 4.2.

Results

The weather conditions were quite favorable during transplanting and consequently little transplanting stress was observed even with standard management practices.

4.3.1 Tomatoes

Stand establishment for the tomatoes was 100% in all treatments. Unlike last year, no visual chlorosis and phytotoxic effects was observed in the 2000 trial. This reflects the refinement of the treatments based on the last year's results. ABA treated plants had, on average, greater shoot weights than the control (Table 4.3.1). Total fruit yield (P>F= 0.08) and total marketable fruit yield (P>F= 0.07) were also higher for ABA treated plants than for the controls. This suggests that foliar applied PBI 365 at relatively lower concentrations enhanced shoot growth, fruit set and development. The effects of ABA concentrations and times of application were insignificant for all the parameters observed in this study.

Table 4.3.1: F test probabilities and treatment means for single degree of freedom contrast between control and ABA treatments for agronomic and yield parameters of tomato treated with varying concentrations of PBI 365 as foliar spray.

Trt. Contrast	Total marketable yield (kg/plant)	Total yields (kg/plant)	Total ripe yield (kg/plant)	Shoot weight (kg/plant)
Control vs. ABA				
Control	5.5	8.81	2.35	2.09
ABA	6.26	9.26	2.18	2.55
F value	3.35	3.13	0.01	6.87**

4.3.2. Pumpkin

These results were not available by the time of reporting

Discussion

Foliar application of PBI 365 at different concentrations and installments had no obvious effect on transplant survival of tomato seedlings under management practices designed to minimize crop stress. Yield effects of the selected ABA treatments were either neutral or positive. This suggests that the treatment concentration selected was relatively safer than the concentrations utilized in past studies. In this study, enhanced shoot growth in the ABA treated plant replicates the superiority of some ABA treatments observed in past studies. This enhanced growth might be due to greater tolerance of the transient and mild stresses inevitably encountered during field production.

As the duration of efficacy of foliar applied ABA treatments was considerably shorter than when the product was applied as a root drench, each concentration of the chemical used in the treatment was repeated two times at an interval of one week each in this trial. However, we could not detect any difference between different concentrations and installments of treatment application. These results suggested that application of PBI 365 at lower concentrations had no detrimental effects on transplant performance under non- stressed conditions.

Conclusion and Suggestions

This study demonstrated that the application of ABA analog (PBI 365) to tomato seedlings prior to transplanting had no detrimental effects on survival growth and yield potential as observed in past studies. Thus, foliar sprays of PBI 365 could be used as a standard pre-transplanting treatment to prevent the transplanting stress likely to occur due to unpredictable weather conditions. This treatment would help insure establishment of a good plant population thereby increasing yields in commercial scale tomato production.

We could not detect any differences between different installments of application under non- stressed conditions of field. This effect should be evaluated , under stressed conditions.

Section 4.4 Field Stress Trials

Introduction

Horticultural crops are highly sensitive to environmental stresses such as extremes of temperature and moisture. Plants are particularly susceptible and sensitive to these stresses during transplanting and early establishment. Field conditions during the spring in Saskatchewan are frequently challenging for production of horticultural commodities, with chilling damage and drought stress, the common problems.

ABA is known to be involved in plants stress physiology. This hormone acts as a signaling molecule in various physiological processes including responses to environmental stresses such as drought, cold, salinity and heat (Abrams, 1999). ABA seems to coordinate the overall physiology of plants either by suppressing or promoting processes depending on the circumstances (Addicott, 1983). ABA influences plants' drought and chilling response mechanisms by reducing transpiration water loss through stomatal closure. ABA also regulates the expression of the drought-induced genes (lea type) involved in plants' adaptation to environmental stresses (Bray, 1997). Levels of ABA in plants can be increased through exogenous application. Treatment with can protect crops from desiccation by reducing transpirational water loss during drought stress (Abrams, 1999). Exogenous application of ABA significantly increased stomatal resistance and water potential of pepper seedlings thus reducing transplant shock in greenhouse and field experiments (Berkowitz and Rabin, 1989). Similarly application of ABA to the cut ends of leaf petioles of *Phaseolus vulgaris* and sugarbeet caused rapid stomatal closure within eight to nine minutes (Kriedemann, 1972). Stomatal water loss at transplanting was significantly reduced bare- rooted Brussels sprouts seedlings treated with ABA (Mc-Kee, 1978). Increased freezing tolerance, low temperature growth and advanced flowering were observed in ABA treated *Brassica napus* and *Brassica compestris* seedlings (Wilen et al. 1994).

The protective effects of exogenously applied ABA are very short lived due, in part, to poor uptake and rapid turn over in the plant systems (Abrams *et al*. 1997). To address this problem, chemically synthesized analogs of ABA have been developed which mimic the effects of ABA (Abrams *et al*. 1997). Alteration in the ABA structure cause the analogs to be metabolized more slowly than ABA, resulting in longer lasting and more effective biological activity (Abrams *et al*. 1997). Thus, ABA analogues have potential as crop protection chemicals, particularly for drought stress. The antitranspirant effects that the ABA analogues produced on barley leaves and water stressed cotton plants were found comparable with that of ABA (Carbonmier *et al*. 1981). Grossnikel *et al*. (1996) reported improved survival of ABA analog treated spruce seedlings under conditions of water stress in the field. ABA analogues were found to increase chilling as well as freezing resistance in a number of crops plants including maize, wheat, rice, cotton, cucumber (Dorffling *et al*. 1989). It appears that the ABA analogs trigger stomatal closure, which reduced water loss during chilling period. Similarly, a number of ABA analogs applied as a root drench to rye seedlings enhanced freezing tolerance and improved root growth (Abrams, 1999).

In greenhouse trials reported elsewhere in this document application of of ABA analogues to tomato and pumpkin transplants was shown to slow water use and delay the onset of drought stress. However, the effects of the synthetic hormone on drought tolerance, plant growth and development were analog and crop specific and varied with the mode of application, concentration of chemical applied, under both stressed and non-stressed sets of experiments. Root dip treatments were more effective than foliar application in terms of drought protection. However, since root dip treatments utilized much more chemical and appeared to be more phytotoxic in some cases, multiple foliar applications of ABA were considered as a possible option. These studies indicated that applying the ABA analogs at 10-4 M concentration provided good stress protection without causing any significant phytotoxic effects.

This study was designed to evaluate the effects of ABA analogues (PBI 429 and BI 365) against the types of stresses encountered in the field following transplanting. A series of field trials were conducted over the 2000 growing season in order to study the various combinations of stresses that are likely to occur during transplanting in a particular year. The study evaluated the comparative efficacy of mode of application and analogue type to identify the appropriate combination of analogue type, mode of application and timing of application. Parallel trials were conducted in June, July and September. The June trial was designed to evaluate the impact of the ABA treatments under the types of stress typically experienced in the spring (cold and wind). The July trial was designed to test seedling performance under hotter conditions while the September trial was designed to test seedling performance at a time when frost was likely. Only yhte data for the June trial is included in this report.

Materials and Methods

Tomato (*Lycopersicon esculentum* cv. Manitoba) seeds were seeded in the Department of Plant Science greenhouse during first week of May 2000. Seeds were planted into 85 cm^3 cell packs containing Sunshine Mix 4 (Sun Gro Horticulture Canada). Greenhouse conditions were $23^0C/18^0C$, day/night temperature with 14-hour daylength without supplemental light. The seedlings reached an appropriate size for transplanting four weeks after seeding. The seedlings were treated with 8' methylene ABA (PBI 365) and 8' acetylene ABA (PBI 429) solutions (10-4M) in 1% acetone two days before transplanting into the field. The treatments were applied a root dip or as a foliar spray. The concentration selected was the most promising concentration identified from previous studies with ABA analogues by the Department of Plant science at the University of Saskatchewan. A solution containing water and 1% acetone was used as control treatment. A summary of the treatments is presented below.

T1 - PBI 365 10^{-4} M foliar spray
T2 - PBI 365 10^{-4} M root dip
T3 - PBI 429 10^{-4} M foliar spray
T4 - PBI 429 10^{-4} M root dip
T5 – Control foliar
T6 - Control root dip

In the root dip treatments, the plants were immersed in the appropriate concentration of ABA solution for two minutes. In the foliar applied treatments, an atomizer was used to apply the spray to leaf run off. No spreader/ sticker was used. On average, 10-ml of the ABA solution was applied to each seedling as a foliar spray and 40-ml was absorbed in the root drench treatment. The seedlings were planted at the Plant Science Department Horticulture Field Research Station at the University of Saskatchewan in Saskatoon. The site is sheltered and features a Sutherland series heavy clay soil. The field was prepared prior to planting by discing and roto-tilling and recommended amounts of fertilizer were incorporated during the tillage (100 and 120 kg/ha of N and P respectively). Treatments were arranged in a Randomized Complete Block Design with four replicates per treatment. Each treatment replicate consisted of eight plants. The in-row plant spacing was 50 cm with 2 m between rows. Transplanting was done on June 5.

The weather conditions during and following transplanting were hot and dry. The volumetric soil moisture content at transplanting was 45%. **No irrigation was applied following transplanting.**

Visual observation on the severity of wilting were made daily (in the afternoon) after transplanting. Leaf temperatures were recorded two days after transplanting when all the control plants were wilted. At the termination of the trial, the number of plants and number of flowering plant per treatment, plant height, shoot fresh weight and dry weight per plant were measured.

Data were analyzed using the GLM program of SAS. Where F values indicated significant (P=0.05) treatment responses, orthogonal contrasts (P = 0.05) were used for comparison of the treatment effects. LSD tests (P=0.05) were used for comparison of treatment means. The following planned single degree of freedom contrasts were made:

ABA treated vs. Control
Analog type (PBI 365 vs. PBI 429)
Foliar vs. root application
Analog type* mode of application

Results

The weather conditions during and following transplanting were hot and dry. Consequently the conditions were favorable for development of stress and the evaluation of treatment effects on drought tolerance and transplants survival. The volumetric soil moisture content at transplanting was 45%. **No artificial irrigation was applied following transplanting.** A light rain shower (0.84-cm) occurred on the fourth day of transplanting. The average air temperature on the day of transplanting was 20.54 °C (min 9.42 °C and maximum 31.12°C). The average air temperature for the first week of transplanting ranged from 20.92 °C to 7.31°C while the average soil temperature ranged from 12.55 °C to 33.39 °C.

Significant drought stress and transplanting shock were observed a day after transplanting and onwards. The control plants exhibited severe transplanting shock within a day of transplanting, as did all the plants treated with PBI 365 as a foliar spray.

Disparity of temperature between air and leaf

Disparity of temperature between leaf and air is an indicator of severity of moisture stress in plants. Under normal conditions, the leaf temperature is close to the ambient temperature. When the plants experience water deficit, the leaf temperature increases above the ambient temperature depending upon the severity of the water deficit.

The disparity of temperature between leaf and air measured on the second day of transplanting varied significantly among the treated and control plants. The mean difference in temperature was significantly greater for control plants than those treated with ABA (Figure 4.4.1). The analog type and mode of ABA application also

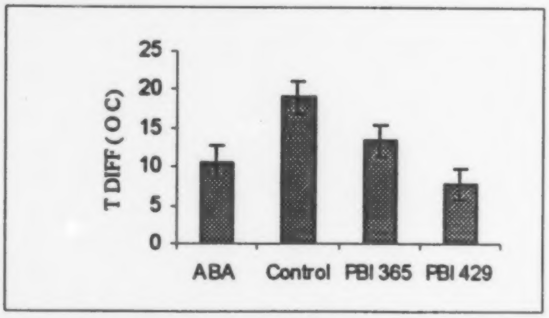


Figure 4.4.1: Temperature difference between air and leaf for tomato plants treated with two ABA analogs two days after transplanting.

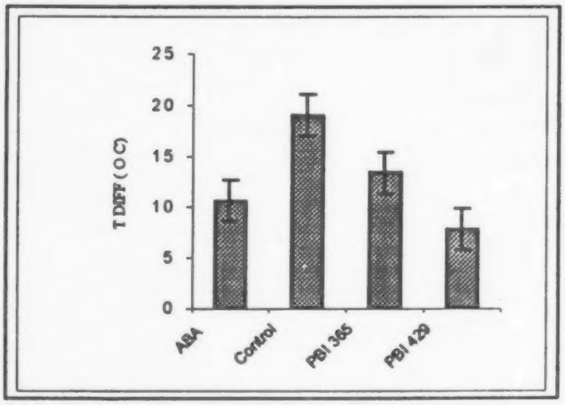


Figure 4.4.2: Temperature difference between air and leaf of tomato plants receiving foliar and root dip treatments of two ABA analogues (PBI 365 and PBI 429) two days before transplanting.

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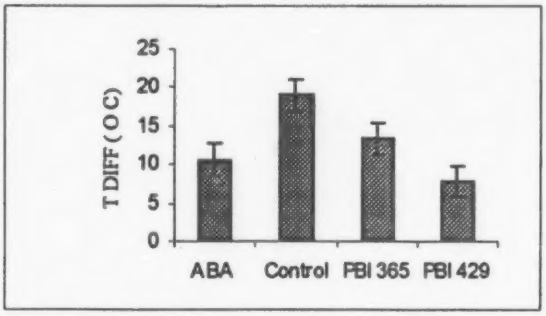

Figure 4.4.1: Temperature difference between air and leaf for tomato plants treated with two ABA analogs two days after transplanting.

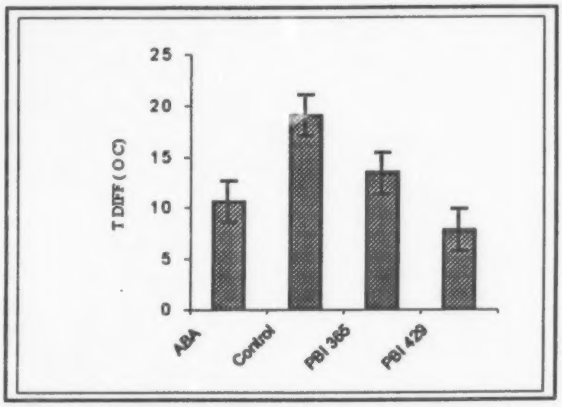

Figure 4.4.2: Temperature difference between air and leaf of tomato plants receiving foliar and root dip treatments of two ABA analogues (PBI 365 and PBI 429) two days before transplanting.

had a significant effect on this parameter. PBI 365 and foliar applied treatments, on average, recorded higher mean differences compared to PBI 429 and root drench treatments respectively (Figure 4.4.1 and 4.4.2).

Transplant survival

On average, 81.25% of plants treated with ABA survived through to the conclusion of the trial compared to only 53.12% survival for the control (Figure 4.4.3). Analogue type and mode of ABA application showed a highly significant difference in terms of final stand count (Figures 4.4.3). There was a significant interaction between analog type and mode of treatment application. Irrespective of the mode of application, PBI 429 recorded higher mean percentages (98.44%) for plant survival compared to PB 365 (65.62 %). The root drench treatments recorded 100% survival against 64% for the foliar applied treatments.

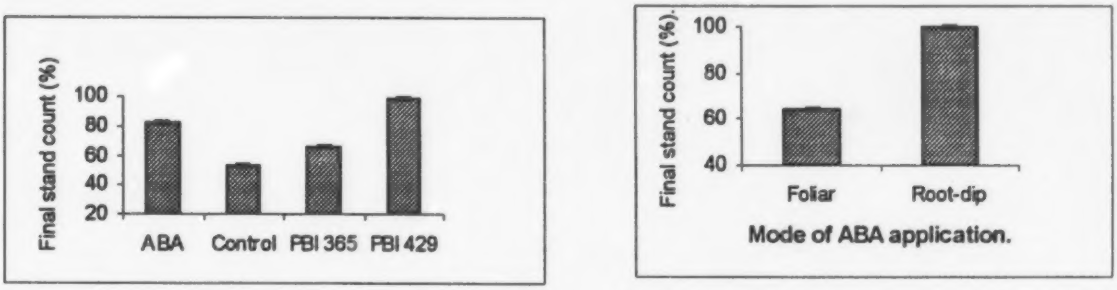

Figure. 4.4.3. **Mean final stand count percentages for tomato transplants treated with ABA analogues (PBI 365 and PBI 429) either as foliar spray or root dips. (Vertical bars represent standard errors).**

Flowering

Compared to the control, the ABA treatments had no significant effect on plant development and flowering. Neither there was significant difference between mode of ABA application. However, the type of analog applied had a highly significant effect on flowering. PBI 429 treatments delayed flowering as compared to PBI 365 treatments (Table 4.4.1). The interaction between analog type and mode of application had a highly significant effect on flowering. By the end of the trial, 93.75% of the plants given root applied PBI 365 flowered where as PBI 429 did not produce flowers. This suggests that root applied PBI 429 (10^{-4} M) retarded plant growth and development, although this treatment provided 100% protection against transplanting stress. Among the treatments, root applied PBI 365 recorded highest flowering percentages while foliar applied treatments of both analogs were statistically equivalent to the control. Delayed resumption of plant growth in the treatments that suffered a considerable to severe transplanting stress might have caused a lower treatment mean in terms of flowering.

Plant height

Plant height measured at the termination of the trial was not significantly affected by the ABA treatments. However, there was a effect of analog type (*P*>f = 0.09) and mode of application (*P*>f = 0.00) on plant height. Root drench treatments averaged over the analogs significantly reduced the plant heights compared to the foliar applied treatments. Similarly, PBI 429 treatments, irrespective of the mode of application produced lower mean values for plant heights than PBI 365 treatments (Table 4.4.1).

Shoot fresh and dry weight

Shoot fresh weight at the harvest was significantly for the ABA treated plants compared to the controls (Figure 4.4.4). No differences in shoot fresh weight were evident between the analog types. The mode of treatment application and the interaction between analog tpye and mode of application had significant effects on the mean shoot fresh weight. In general, root applied PBI 365 produced the highest mean shoot fresh weight amongst the treatments while other treatments were similar to control (Table 4.4.1). Shoot dry weight was similarly affected by the ABA treatments (Table 4.4.1).

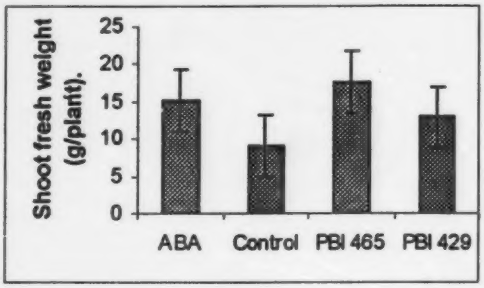

Figure 4.4.4: Mean shoot fresh weight of ABA analog treated tomato plants four weeks after transplanting.

Discussion

Previous greenhouse studies had indicated the potential for using ABA to slow water use of vegetable crop transplants and hence delay the onset of drought stress (Section 1 and 2). Improved transplant tolerance to drought stress and field establishment vigor was also reported in paclobutrazol treated tomatoes (Sauza-M et al. 1999). In this study, application of ABA analogue two days before transplanting in tomato plants increased their tolerance to drought stress through. Protective effects of ABA analogues varied with the mode of application and analog type. Root applied treatments of both the analogues performed better in terms of drought protection than the foliar applications at the same concentration. However, the amount of chemical consumed per root dip treatment was almost five times the foliar application. The differences in relative efficacy of the two modes of application were also frequently observed in Greenhouse trials with pumpkin and tomato. From a grower's perspective, higher amount of chemical used in the root dip treatments increases cost of application. Comparison of root dip treatments against foliar application at different installments at the same concentration would provide a viable solution. Root dip applications at relatively lower concentrations could also be examined as a possible option.

In previous Greenhouse trials, tomato plants treated with PBI 429 maintained their turgidity longer than those treated with PBI 365 (Section 1). In this study, PBI 429 treatments again showed greater efficacy in terms of drought tolerance. However, the growth and development of transplants treated with PBI 429 was significantly retarded compared to the PBI 365 treatments. Root applied PBI 429 (10-4M) appeared to be somewhat phytotoxic as indicated by a degree of chlorosis and severe retardation of top growth. PBI 429 root dip treatments were relatively more phytotoxic than PBI 365 in past greenhouse studies (Section 1). In spite of significant effect of analog type and mode of application on plant survival, growth and development, the ABA treatment had a little effect on final shoot biomass yield.

Conclusion

This study suggested that ABA analogues could be potentially used in transplanted horticultural crops like tomato to reduce the risk of moisture stress and transplanting shock. Root dips containing PBI 429 and PBI 365 at 10-4M slowed the moisture loss from tomato transplants thereby increasing drought stress tolerance and avoiding transplanting shock. Foliar applied treatments with the same concentration of the two analogs were relatively ineffective. PBI 365 seems to be less effective than as a means of reducing transplanting shock but it is also less phytotoxic. PBI 429 applied as root dip at 10-4M is not an appropriate treatment as it caused severe retardation of plant growth. The treatment which provided 100% protection against moisture stress without substantially impeding the seedling growth and development was root applied PBI 365 (10-4M). Therefore, from a grower's perspective, this treatment could help to ensure good stand establishment without compromising on subsequent plant growth and yield.

The relatively higher amount of chemical involved in the root dip application increases the cost of treatment application. To address this problem, we should compare root dip treatments against foliar application at different relevant installments at the same concentration, which utilizes almost five-fold less chemical.

Table 4.4.1. Treatment means and sums of squares for plant survival, stress tolerance and growth characteristics of tomato seedlings treated with ABA analogues via foliar or root dip applications two days before transplanting.

Treatments	$^{x}T_{Diff}$(°C)	^{y}% stand count	^{y} SFW (g/plant)	^{y} SDW (g/plant)	^{y} % flowering	^{y} Plant height (cm)
PBI 365						
1 x10^{-4} M Foliar	18.75	31.25	8.76	1.46	25	19.53
1 x10^{-4} M Root	8	100	26.16	4.84	93.75	19.17
PBI 429						
1 x10^{-4} M Foliar	11.75	96.87	16.85	2.41	44.5	20.63
1 x 10^{-4} M Root	4	100	8.91	1.38	0.00	16.34
Control Foliar	20.75	37.5	9.29	1.59	17.75	19.68
Control Root	17.25	68.75	8.97	1.26	45	18.68
LSD (0.05p)	**4.39**	**10.98**	**8.77**	**1.30**	**34.29**	**1.48**
Treatment SS	870.83	129.20	1010.65	14.89	20900.70	42.42
Contrast SS						
ABA vs.control	374.08	0.31	194.64	3.07	481.33	0.38
365 vs. 429	121.00	0.28	84.90	1.31	5476.00	2.97
Foliar vs. root	342.25	0.34	89.58	0.99	576.00	21.62
Analog*mode of application	9	0.28	642.11	9.28	12882.25	15.44

x; data collected two days after transplanting, y; data collected at termination of the trial (a month after transplanting).

Section 5. Use of ABA Analogues for Control of Growth and Diseases of Potatoes

Trial 5.1. ABA Analogues for the Control of Common Scab (*Streptomyces scabies*) in potatoes.

Potatoes are the most widely grown Horticultural crop, with > 160,000 acres seeded to potatoes in western Canada in 1998. Although potatoes are generally well adapted to production on the Canadian Prairies, the crop is sensitive to a range of diseases. Common scab caused by *Streptomyces scabies* is one of the most common problems affecting the industry. Losses to scab are primarily due to its adverse effects on tuber appearance. In any year, 10-20% of the table stock fields grown in Saskatchewan can have scab levels above commercial tolerance levels resulting in high grade outs and reduced crop quality. *Streptomyces* occurs in almost all soils and cannot be effectively controlled via crop rotation. Greatest problems with scab occur in soils with pH between 5.6 and 7.5, especially if the land has been manured or has a heavy trash load. These soil pH levels are typical of the Canadian Prairies. At present, there are no effective seed or foliar applied chemicals for control of soil-borne scab.

The incidence and severity of common scab in potatoes is thought to be related to drought stress early in the development of the tubers. Although growers may strive to minimize this stress through careful moisture management, problems with scab are still common. **In preliminary studies conducted by the Horticulture Science Department the relationship between moisture stress and the incidence of scab by using plant growth regulators to manipulate crop moisture use.** One of the treatments involved application of Abscisic acid at tuber set (15% s-ABA applied at 55 ml/a). ABA slows growth and reduces water use by temporarily closing the stomata. By using ABA to slow crop water use at the crucial period for the onset of scab it tuber it was hoped that problems with this disease could be reduced. In trials conducted in 1995 and 1996, foliar applied ABA reduced the incidence of scab but did so at the expense of overall yields.

This trial evaluated the potential to utilize the more bio-active ABA analogues in scab control in potatoes.

Trial Description

The 1998 ABA trial in potatoes was conducted on the Horticulture Science Department Potato Research plots located in Saskatoon. The site features a sandy loam, pH 7.2, EC < 1 dS, with 4% O.M. The site has been cropped to potatoes on a 3 year rotation with dryland barley for the past 20 years. The plots receive an application of 20 t/ha of manure every 3 years. **Common scab has been a consistent problem on the site.**

Production Practices

Unless otherwise specified standard production practices for irrigated commercial potatoes were used in this trial. The field was double disced prior to planting and sufficient N as 46-0-0 was applied at this time to raise soil N levels to the 150 #/a recommended for irrigated potatoes. The crop was seeded in mid-May, using single cut or drop type seed treated with Polyram (metiram) as a seedpiece treatment. The crop was seeded at 23 cm intervals in rows 90 cm apart. Sufficient phosphorus (11-55-0) was applied in a band adjacent to the seed to raise soil levels to 120#/a. The test cultivars were;

> Norland - an early maturing red skinned table type with moderate resistance to common scab. Shepody - a leter maturing processing variety with limited resistance to scab.

Weed control was achieved through pre-plant application of Eptam (EPTC) and Sencor (metribuzin) followed by Gramoxone (paraquat) and Lorox (linuron) at ground crack with Poast (sethoxydim) applied 2 weeks later. A rolling cultivator was used at ground crack for inter-row tillage and to throw hills. The plots were irrigated using a wheel-line system. Soil moisture levels were monitored in the plots using tensiometers or electrical resistance metres. The plots were irrigated whenever soil water potentials rose above -50kPa.

The crop was treated on July 6. Norland had just begun to set tubers at this time while the slower growing Shepody had only reached the hooking stage (stolons terminate in a hook which develops into the tuber). The crop was treated at this time as it is at this stage that the scab organism attacks the newly developing tuber. The plants were sprayed with 5x10-5 M solutions of either PBI 365 or PBI 429. This rate represents a mid-point in the concentration range used in preliminary trials conducted with pumpkin and tomato seedlings. The spray was applied using a CO2 power backpack sprayer calibrated to deliver 100 l of spray/a at 600 kPa. A single row was sprayed in each plot with each analogue - treated rows were separated by a non-treated guard row.

No significant problems with insects or diseases were observed and the plots received no pesticides beyond those already described. The crop was desicated using Regalone (diquat) in early September and harvested two weeks later. The crop was graded into size categories; small = < 44 mm diam., 44 < medium < 88 mm and oversize = > 88 mm diam. A sample of 100 tubers from each treatment were evaluated for the presence of scab and the percentage of total tuber area covered with lesions. According to Agriculture grading standards, potatoes meet Grade 1 standards if less than 10% of the surface is affected by scab lesions. A final grade assessment determined what proportion of the crop met Grade 1 standards for both size and freedom from excessive scab.

1998 Growing Season

The mild winter and early dry spring accelerated soil warming and allowed for earlier than normal planting in 1998. The remainder of the 1998 growing season was unusually warm and dry. Virtually all the crop water requirements had to be fulfilled through supplemental irrigation. Crop health and vigor was excellent through until top-kill.

Results

The ABA sprays produced no visible changes in the appearance or development of the crop. Yields of both cultivars were substantially higher than provincial norms (Table 5.1). This reflects the excellent growing season and the high level of management employed in the trials. As expected, the incidence and severity of scab was much higher for cv. Shepody than for cv. Norland (Table 5.1). The ABA treatments had no impact on yields or grade out to scab for cv. Norland. For cv. Shepody, both ABA treatments significantly enhanced yields relative to the control. The yield enhancement occurred in all tuber size categories. The ABA treatments had no impact on losses to scab and yields after grade out were comparable for all treatments.

Conclusions

Application of ABA analogs at tuber initiation had little effect on scab or marketable yields in this trial. These results do not correspond with previous years where ABA applied to the foliage at tuber set had reduced yields but also had reduced grade out to scab. In other trials conducted in 1998 (see next section) foliar applied ABA analogs again produced few effects on growth or yields of potatoes. It is possible that the concentration of ABA

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selected (10-5 M) for application is not appropriate for potatoes. This rate was selected as it represented the mid-point in the concentration range evaluated in screening trials conducted on pumpkin and tomato seedlings. ABA applied to the foliage at 10-5 M to these crops had moderate effects on crop water relations (see Sections 1 and 3), but the effects was not as powerful as when the ABA was applied as a root drench.

Recommendations

a) Screening trials of different ABA concentrations should be conducted.
b) Once effective rates have been identified, the impact of timing of application should be examined.

Table 5.1. Yield and scab severity for two cultivars of potato as influenced by foliar applied ABA analogs.

	NORLAND			SHEPODY		
	Marketable Size (t/ha)	Gradeout %	Marketable (t/ha)	Marketable Size (t/ha)	Gradeout %	Marketable (t/ha)
Control	55.5	33	34.4	40	83	5.6
PBI 365	59.4	41	33.2	45.3	83	6.7
PBI 429	62.3	32	39.8	45.8	91	3.9
LSD (0.05)	NS	NS	NS	4.6	NS	NS

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PBI 429	62.3	32	39.8	45.8	91	3.9
LSD (0.05)	NS	NS	NS	4.6	NS	NS

Trial 5.2. RESPONSE OF SHEPODY POTATOES TO FOLIAR APPLIED ABA

- 1998 and 1999 FIELD TRIALS -

Western Canada presently grows approx. 140,000 acres of potatoes, with significant expansion of the processing sector driving further growth in the near future. Potatoes are a high value, management intensive crop. One of the major challenges facing growers is control of tuber number and size distribution. Several of the most commonly grown cultivars tend to produce relatively low numbers of tubers. This limits yield potential and causes problems during processing as the tubers tend to become excessively large.

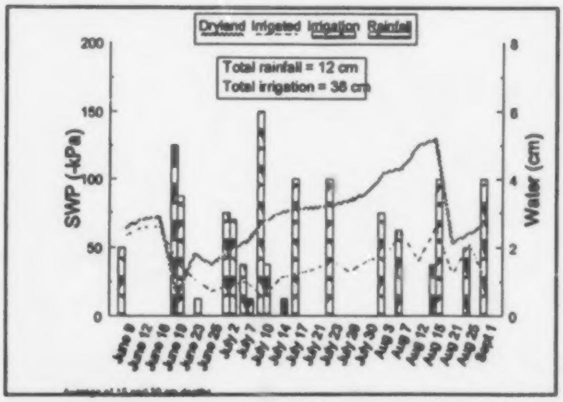
From the seed growers perspective a smaller average tuber size is also desirable as it tends to increase the number of sprouts produced by each seed piece.

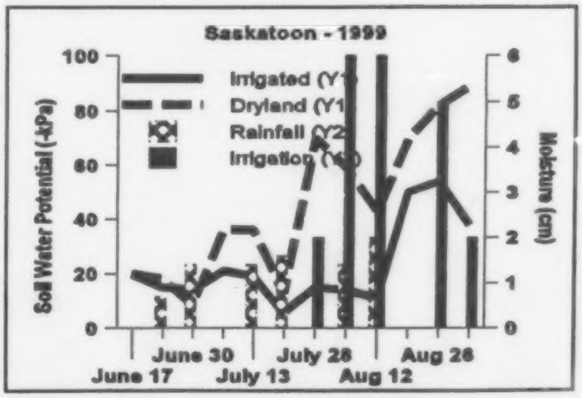
Abscisic acid is a well researched plant growth regulator which controls many aspects of plant development and physiology. In potatoes, tuber set and development is trigger by complex shifts in the levels of various plant growth regulators. Application of natural ABA has been shown to alter tuber set and size distribution in both tissue culture and field trials. The enhanced efficacy and duration of impact shown by the ABA analogues could be useful in management of tuber size. ABA is also responsible for the onset and maintenance of dormancy in many plant species and structures. Use of ABA analogues as an enhancer of natural dormancy would be of great use to the seed potato industry in Canada.

This study examined the potential for using foliar applications of abscisic acid (ABA) analogues; as a means of enhancing tuber set and yields of potatoes grown under irrigated conditions.

Trials were conducted in 1998 and 1999 at the Plant Science Department Potato Research Plots in Saskatoon. The land is a sandy loam (pH 8.1, EC <1 dS/m, 3% organic matter content). The land has been cropped to potatoes one year in every three for the last 20 years. Barley is used as the alternate crop. The cultivar tested was Shepody which is a mid-season cultivar commonly grown for processing. This cultivar has relatively high yield potential but sets very few tubers.

The crop was planted in mid-May using Elite 3 seed which was cut, treated with mancozeb and planted at 30 cm intervals within the row with 1 m between rows. Soil fertility levels were adjusted prior to planting to correspond with recommendations for irrigated potatoes (150 kg/ha N, 120 kg/ha P2O5). Weeds were controlled by pre-plant application of Eptam (EPTC) and Sencor (metribuzin) with Gramoxone (paraquat) and Lorox (linuron) applied at ground crack and Poast (sethoxydim) applied just prior to row closure. The crop was hilled just prior to and





two weeks after emergence. Soil moisture potentials at 15 cm were monitored using tensiometers (see **Figure 5.2.1 and Figure 5.2.2 for 1998 and 1999 profile**). In 1998 a total of 12 cm of rainfall fell during the cropping season. This represents 62% of normal precipitation. An overhead irrigation system was used to maintain soil water potentials in the plot at recommended levels (above -50kPa). In 1998 a total of 38 cm of water was applied during the cropping season. The 1999 cropping season was considerably wetter and cooler than 1998 - consequently less total water (34 cm) and less supplemental irrigation (21 cm) was required.

The crop was sprayed with 5x10-5 M 8' acetylene (PBI 429) or 8' methylene (PBI 365) ABA on July 1, August 1 or September 1. This concentration was selected as representing the mid-point of the ranges tested in the preliminary trials. It therefore presented a reasonable expectation of crop response and did not represent an excessively costly treatment option. The earliest spray date coincides with the onset of tuberization, the mid-season date corresponds with tuber bulking while by the final date the crop should be maturing. Plants sprayed with water and the 1% acetone used as the solvent for the ABA served as the controls.

ABA treatments were arranged in a randomized complete block design with three replicates. Each plot consisted of a single 8 m long row of potatoes separated from adjacent rows by a non-treated check row.

The field was top killed in mid-September and harvested three weeks later.

The crop was graded into the following size categories; small = < 33 mm, marketable = 33-80 mm and oversize = > 80mm. Tuber weights and counts were determined for each category. Tablestock yields included all tubers > 33mm in diameter, while seed yields were all tubers < 80 mm in diam.

Data from the trial was analysed using the GLM procedures of SAS. Single degree of freedom contrasts (P=0.05) were used to examine;
a) ABA treated versus control
b) effect of time of application of ABA
c) 8' acetylene (PBI 429) vs 8' methylene (PBI 365) ABA

1998 Results

The 1998 growing season was almost ideal for production of irrigated potatoes. Spring conditions were favorable and July and August featured long stretches of very warm weather. Fall frost was delayed until well into September.

The statistical analysis for the ABA treatment effects on yields and tuber size distribution of the Shepody potatoes in 1998 are presented in Table 5.2.1, while the treatment means are presented in Table 5.2.2. No treatment effects approached statistical significance for any of the yield parameters examined (Table 5.2.1). The coefficient of variability for the treatment means of most of the yield variables was in the range of 20% which is fairly high even for inherently variable crops such as potatoes. However, given the similarity of the treatment means (Table 5.2.2) it seems unlikely that any real treatment effects were obscured by experimental variability or the limited number of replicates used.

Table 5.2.1. **F test probabilities for single degree of freedom contrasts for yield components of Shepody potatoes treated with 8' methylene (PBI 365) or 8' acetylene (PBI 429) ABA at various times during the 1998 growing season.**

	Total Weight	Tablestock	Seed	Average Tuber Wt.
ABA vs NON	0.77	0.75	0.37	0.78
PBI 429 vs 365	0.58	0.56	0.31	0.91
ABA Timing	0.86	0.75	0.96	0.46
Timing * ABA form	0.73	0.69	0.8	0.26
CV%	20%	21%	17%	13%

Table 5.2.2. **Treatment means for yield components of Shepody potatoes treated with 8' methylene (PBI 365) or 8' acetylene (PBI 429) ABA at various times during the 1998 growing season.**

	Total Weight	Tablestock	Seed	Average Tuber Wt.
		t/ha		(g)
Control	40.7	38	37.8	237
PBI 429	40.3	37.6	35.8	232
PBI 365	38.2	35.4	32.8	231
LSD (0.05)	9.6	9.6	7.3	44
Early	38.7	35.8	33.5	232
Mid-Season	39.7	37.3	34.4	239
Late	39.3	36.3	34.6	223
None	40.7	38	37.8	237
LSD (0.05)	*10.3*	*10.1*	*7.7*	*45*

PBI 429 = 8' acetylene, PBI 365 = 8' methylene

1999 Results

The 1999 growing season was relatively cool and wet. Spring conditions favored development of several diseases. The dat of fall frost was close to the 30 year norm.

The statistical analysis for the ABA treatment effects on yields and tuber size distribution of the Shepody potatoes in 1999 are presented in Table 5.2.3, while the treatment means are presented in Table 5.2.4. Contrasting the

ABA treated crop versus the controls, the only significant difference was that ABA treatment, on average, reduced tuber size relative to the control, without negatively impacting yields. The 8' methylene analog (ABA 365) actually reduced yields somewhat - this is reflected in the significant single degree of freedom contrast of ABA 365 vs 429. Applying ABA 429 on July 1 appeared to have the greatest positive effect on tuber set - but the results were still not significantly different than the control (Table 5.2.4). The coefficient of variability for the treatment means of most of the yield variables were lower than in 1998 but were still fairly high even for inherently variable crops such as potatoes.

Table 5.2.3. **F test probabilities for single degree of freedom contrasts for yield components of Shepody potatoes treated with 8' methylene (PBI 365) or 8' acetylene (PBI 429) ABA at various times during the 1999 growing season.**

	Total Weight	Tablestock	Seed	Average Tuber Wt.
ABA vs NON	0.27	0.24	0.7	0.04
PBI 429 vs 365	0.02	0.03	0.12	0.8
ABA Timing	0.46	0.75	0.96	0.46
Timing * ABA form	0.73	0.69	0.8	0.26
CV%	12%	13%	13%	9%

Table 5.2.4. **Treatment means for yield components of Shepody potatoes treated with 8' methylene (PBI 365) or 8' acetylene (PBI 429) ABA at various times during the 1999 growing season.**

	Total Weight	Tablestock	Seed	Average Tuber Wt.
		t/ha		(g)
Control	46.2	43.6	40.5	248
PB! 429				
- July 1	46.2	41.3	42	214
- Aug 1	46.8	44	41.2	237
- Sept 1	44.2	40.9	40.8	222
PBI 365				
- July 1	40.6	38.2	36.8	227
- Aug 1	40.5	37.5	37.6	230
- Sept 1	39.6	35.2	37.2	207

PBI 429 = 8' acetylene, PBI 365 = 8' methylene

Conclusion - Foliar application of ABA 429 or 365 at 5X10-5 M at three dates during the growing season caused no obvious changes in the morphology, development, yields or tuber size distribution of Shepody potatoes during two years of testing.

Section 6. Impact of ABA analogs on Tuber Dormancy of Potatoes.

ABA levels are known to govern seed dormancy of many crops. ABA is also involved in the dormancy of potatoes, although the relationship is complicated by the effect of other plant growth regulating chemicals. Prolonging the dormant period of potatoes is desirable in both seed and table potatoes as pre-mature sprouting accelerates moisture loss from the tubers and triggers undesirable changes in the chemistry of the tubers. In table stock and processing potatoes, sprouting is control via the application of chemicals such as CIPC. These chemicals kill the sprouts and are therefore not suited for use on seed potatoes. In seed potato production, low temperature storage is the only means presently available for controlling sprouts. Unfortunately, excessive sprout growth is a common occurrence in the seed industry.

Jeff Suttle of the USDA has been using ABA treatments to extend the dormancy of potato micro-tubers. Preliminary results indicate that dunking the tubers in solutions containing either natural ABA or ABA analogs extended the dormancy with no longterm negative effects on the viability of the tubers as seed. In this study, we sought to evaluate the potential for using ABA analogs to extend the dormancy of seed potatoes. **The study examined the impact of ABA rates and analog types applied as a spray rather than as a dip.**

Uniform, wound free tubers harvested in mid-October from University of Saskatchewan field plots and were selected for treatment. Two varieties were tested: Norland which has a short natural dormancy period and Russet Burbank which has a longer dormancy period. The treatments were applied in mid-December by which time the crop had been in cold storage (4C) for nearly two months. This delay in treatment allowed for complete wound healing. There were no indications of either variety breaking dormancy at the time of treatment. Tubers were lightly brushed to remove excessive soil prior to treatment. The potatoes were treated with 8' methylene ABA at $5*10^{-5}$, 10^{-4} or 10^{-3} M. The analogs were applied by spraying the tubers to surface wetness. As soon as the potatoes dried, they were returned to the longterm cold storage (4C and 90% RH). These storage conditions correspond with the standard conditions maintained by growers of seed potatoes. At intervals over the following months, the potatoes were evaluated for sprouting. At each interval a sample was moved from the cold storage into conditions conducive to rapid sprout development (20C in darkness). Sprout growth under these conditions was evaluated to determine the impact of the ABA treatments.

Results

At two months after treatment (Jan 25), there were no measurable sprouts when the potatoes were removed from cold storage. Sprout growth was monitored for the next 8 weeks during which time the tubers were stored under conditions conducive to sprout growth (20C and dark). **There were no significant treatment effects for any of the variables examined (sprout length, number or weight).**

Conclusion - it appears that the spray treatments utilized in this trial had no impact on the dormancy or sprouting characteristics of the potatoes.

Section 7. Potential for using ABA analogs to control growth of recreational turf.

INTRODUCTION

Plant growth regulators (PGR's) represent an important tool in managing plant growth and maintaining plant quality for a wide range of horticultural crops. However, there is little information presently available on the potential for using PGR's to influence the growth, mortality, and reproduction of turfgrass. Interest in using PGR's to suppress the growth of turfgrass is increasing due to rising maintenance costs (McLaren 1982). The ideal PGR for turfgrass would provide prolonged plant growth reduction after application while retaining the cosmetic appearance of the turfgrass (Marshall 1985). The PGR treatment should also be easy to apply and available at a low cost (McLaren 1982).

Hongfei et al. (1998) and Johnson (1994) indicated that PGR's, such as paclobutrazol, have been used to suppress seedhead production in turfgrass growing in low maintenance areas such as highway roadsides, airports, and golf course roughs. This treatment is designed to reduce growth while maintaining the vegetative quality of the turfgrass (McCaughey 1989). In recent years, new chemicals have been designed and developed to use in more intensively maintained commercial turfgrass situations. However, McLaren (1982) indicated that several undesirable characteristics have been associated with these PGR's including: (1) phytotoxicity to treated leaf blades, (2) reduced recuperative potential following physical damage to the treated turfgrass, and (3) increased weed pressure due to reduced competitive potential of treated turfgrass. When PGR's are used in low maintenance areas, these undesirable characteristics do not pose a problem. In situations where the turfgrass is under more intensive use, these PGR effects can have long-term negative side-effects. Long-term growth suppression might adversely affect the potential of the turfgrass to recuperate from summer stresses (Han et al 1998). However, any PGR applications that may suppress vegetative turfgrass growth may also increase photosynthetic assimilate supply to axillary buds resulting in their release thereby promoting growth and developing seeds (Hopkins 1999).

The various responses of turfgrass to any chemical treatments may vary according to environmental conditions, time of application, concentration of the applied product and other local factors. Suppressing the plant growth rate is the main purpose of applying PGR's to turfgrass but other effects could also benefit the producer. Reduced water loss through transpiration or increased resistance to drought stress are commonly reported side effects of treating turfgrass with PGR's (Hongfei et al. 1998). Less irrigation may be needed to maintain visually appealing turfgrass following treatment with PGR's. Less frequent watering would also reduce labor and equipment costs in turfgrass maintenance.

Abscisic Acid

Abscisic acid (ABA) is a phytohormone normally found in low concentrations in all higher plants. ABA appears predominantly in the chloroplasts of mature green leaves and is synthesized mainly by the mesophyll cells (Hopkin 1999). ABA activates intracellular signaling mechanisms thereby regulating the plant's physiological response to environmental stress (Meulen et al. 1993). ABA is rapidly synthesized in response to stress factors including drought, cold, salinity, and heat (Hopkins 1999). Stems and/or roots under physical or moisture stress also produce ABA (Puliga et al. 1996). ABA moves rapidly between regions of the plant (Westwood 1995). In both the xylem and the phloem tissues, transported ABA can be translocated through parenchyma cells (Hopkins 1999). The movement of ABA does not exhibit polarity like the movement of auxins (Westwood 1995).

The following summaries the physiological responses known to be associated with ABA (Hopkins 1999):

- Stimulates the closure of stomata.
- Inhibits shoot growth.

- Either has little effect or will promote root growth.
- Inhibits the effect of gibberellin.
- Induces or maintains dormancy.
- Induces transport of photosynthate toward developing seeds and promotes the synthesis of storage proteins.

ABA synthesis appears to play a major role in regulating stomatal function. The majority of the water taken up by a plant is lost during cellular transpiration and most of this water will escape through the stomata (Roberts et al. 1988). Stomata have a pair of guard cells. When these guard cells are turgid, the stomata are open and water is lost through transpiration. Rising ABA levels in the plant tissues triggers the closure of stomata when soil water supplies are insufficient to keep up with the rate of transpiration (Hopkins 1999). Rising cellular levels of ABA modulate the activity of various ion channels, which alter the osmotic potential of the guard cells and promote collapse of the guard cells. This collapse causes closure of the stomatal space. ABA levels in the guard cells will return back to normal conditions when soil water levels are adequate. The adequate supply of soil moisture will result in stomata opening (Lindgren 1999).

Plant tissues can sense water deficits (Hopkins 1999). Relatively large amounts of ABA are rapidly synthesized in the leaves in response to water stress (Roberts et al. 1988). The roots of many plant species can also sense drought conditions. Under such circumstances, accumulating ABA inhibits auxin-induced shoot growth, but stimulates root growth. The resulting root growth enables the plant to maximize its soil coverage and ability to recover scarce water resources (Lindgren 1999).

The relation between water availability, ABA levels, and activity is well documented. The production of ABA in plant tissues will inhibit stomatal opening in many plant species. This appears to offer a means for regulating the water balance within plant tissues (Westwood 1995). Dodd (1996) showed osmotic stress (0 to -0.06 MPa) and ABA treatment (1×10^{-3} M) applied together significantly reduced the growth of barley. It has been shown that the production of ABA is stimulated by moisture deficits in the soil. Puligia (1996) demonstrated a clear relationship between increased ABA accumulation and reduction in leaf growth of many plant species when there were low levels of soil moisture.

Exogenous applications of ABA have been evaluated on turfgrass and other plant species. The main purpose of applying ABA exogenously is to reduce plant growth by causing the stomata to close. The closed stomata will reduce water loss, gas exchange, and thereby reduce photosynthesis. The main problem with this approach is that ABA treatments are rapidly metabolized when applied exogenously to plant tissues (Cutler et al. 1999). This will result in reduced biological response of the plant tissues to the ABA treatments.

ABA analogs have been developed in an effort to produce more prolonged ABA effects following exogenous application. ABA analogs have a much greater biological activity than the natural ABA hormone and also exhibit a greater biological stability for practical applications (Lindgren 1999). Synthetic ABA analogs are more resistant to catalytic breakdown by endogenous plant enzymes than the naturally occurring ABA form. Montague (1997) showed that ABA treatments (1 x 10^{-5} M and 1 x 10^{-4} M) inhibited the internodal elongation within oat stem segments. Ofir (1998) demonstrated that Poa bulbosa exposed to short day lengths ceased to produce new leaves on the tillers and effectively went dormant. Short-term applications of ABA analogs (0.1 – 1.0 mM) had similar effects on growth but dormancy was transient. Endogenous levels of ABA in the leaf blades and at the tiller base increased after the plants were transferred from short day to long day resulting in effective height control (Ofir 1998). ABA analog treatments were also shown to govern dormancy and germination control in guinea grass seeds (Usberti 1997).

Recently, two newly improved ABA analogs have been developed and are now commercially available. The analog 8'-methylene abscisic acid (ABA-365) involves the addition of methylene double bonded to ABA. ABA-365 is more active, persistent, and has a slower metabolism than standard ABA (Abrams et al. 1997). The analog 8'-acetylene abscisic acid (ABA-429) involves the addition of acetylene double bonded to ABA. ABA-429 has shown to maintain or gain effectiveness over time (Churchill et al. 1998). Suzanne et al. (1997) and Van der Meulen et al. (1993) compared the rate of metabolism using foliar applied ABA-365 analog and ABA-429 analog on plant tissues. The ABA-365 analog was slightly more effective at reducing metabolism, more persistent, and showed a slower breakdown in the plant tissue than the ABA-429 analog.

The objective of this trial was to determine the potential for using foliar-applied ABA analogs as a means to control the growth of turfgrass. The ABA-365 and ABA-429 analogs were studied at various concentrations to determine which treatment was the most effective as a PGR for turfgrass.

Materials and Methods

The trial was conducted over the summer of 1999 at the University of Saskatchewan Horticulture Field Lab located on 14th Street in Saskatoon, Saskatchewan. The turfgrass tested was a well established mixture of fescue species and a lawn seed mix which contained Kentucky Bluegrass, Quantum Leap, Alene, Baron, Creeping Red Fescue, and Chewings Fescue. The trial lasted for 30 days, starting on July 5 and running through to August 4, 1999. The trial was terminated once the fresh weight of the clippings and leaf blade height of all the treatments were consistently equivalent to the control. Measurements of leaf blade heights were commenced once the first few sets of fresh clipping weight showed no apparent treatment responses.

The treatments included a control, the ABA-365 analog, and ABA-429 analog. In the control treatment, only water was applied to the turfgrass. The ABA concentrations tested were based on past research conducted by Dr. Doug Waterer, to determine the effects of ABA analogs on various horticultural crops. The ABA analogs were applied at concentrations of 1×10^{-4} M and 5×10^{-4} M. There were five replications of each treatment. Each plot was 18 meters long and one meter wide. The treatments were applied in 80 L/a of water using a carbon dioxide powered back pack sprayer equipped with two 8002 nozzles.

Prior to the onset of the trial, the plots were fertilized with 34 – 0 – 0, thoroughly watered, and then mowed to a standard height of five centimeters. After the application of the ABA, the following parameters were evaluated weekly: 1) plant appearance, 2) average plant height, and 3) weight of the fresh turfgrass clippings obtained when the plot was mowed to a height of five centimeters. Plant appearance was based on visual criteria from personal observations. Leaf blade height was measured from the base of the leaf blades to the tip of the turfgrass. There were ten replications of the leaf blade height measurements per treatment in each replication. The plots were irrigated to maintain plant growth and plant appearance.

The data were analyzed using the ANOVA and GLM procedures of SAS. The p-values ($p = 0.10$) and least significant difference (alpha = 0.10) tests were used to evaluate treatment effects. The p-values above 0.10 were considered as the probability of a treatment having no significant effect on the turfgrass. The least significant difference values determined if an applied treatment was significantly different from the control treatment. Orthogonal contrasts (alpha = 0.10) were used to make planned comparisons between the treatment classes.

Results

The warmest temperature during the test period was 30.8 °C and the lowest temperature was 5.6 °C. The mean temperature during the test period was 18.3 °C with a mean maximum temperature of 24.4 °C and a mean minimum temperature of 11.7 °C. The total rainfall was 84.4 millimeters. An additional 20 millimeters was supplied as irrigation with the total amount of moisture during this test period as 104.4 millimeters.

Fresh Weight of the Turfgrass Clippings

Treatment means for the influence of the ABA analogs on the fresh weight of the clippings obtained from the test plots at varying intervals from the time of application are presented in **table 7.1**. At no point in the trial did the ABA treatments result in a consistent change in the fresh weight of the clippings relative to the control treatment. However, from 5 to 15 days after the initial application, the ABA-365 treatments produced significantly greater fresh weights of clippings than the ABA-429 treatments. At 15 to 20 days after the initial application, the higher concentrations of both ABA treatments appeared to suppress vegetative growth relative to the lower concentration rate. Averaged over the entire trial, the ABA analog treatments had no significant effect on the fresh weight of turfgrass clippings. The fresh weights of the turfgrass clippings for each treatment are presented in a cumulative graph in **figure 7.1**.

Table 7.1: The effect of foliar-applied ABA analogs on fresh weight of turfgrass clippings at various intervals after treatment

Treatment And Concentration	Days After Application						Average Weight (kg / plot)
	5	10	15	20	25	30	
	Weight (kg / plot)						
Control	1.48	1.11	0.86	0.32	0.51	0.60	0.81
ABA-365 ($1x10^{-4}$ M)	1.67	1.19	0.96	0.37	0.55	0.67	0.90
ABA-365 ($5x10^{-4}$ M)	1.53	1.16	0.91	0.30	0.47	0.57	0.82
ABA-429 ($1x10^{-4}$ M)	1.29	1.01	0.91	0.37	0.53	0.53	0.77
ABA-429 ($5x10^{-4}$ M)	1.40	0.93	0.73	0.35	0.54	0.71	0.78
P – value	0.00	0.00	0.00	0.01	0.01	0.14	0.15
LSD	0.20	0.18	0.10	0.07	0.10	0.12	0.21
	CONTRAST						
ABA analogs vs. control	0.94	0.61	0.56	0.36	0.74	0.64	0.94
ABA-365 vs. ABA-429	0.00	0.00	0.00	0.32	0.58	0.98	0.24
Concentration: [high] vs. [low]	0.84	0.35	0.00	0.05	0.29	0.35	0.60

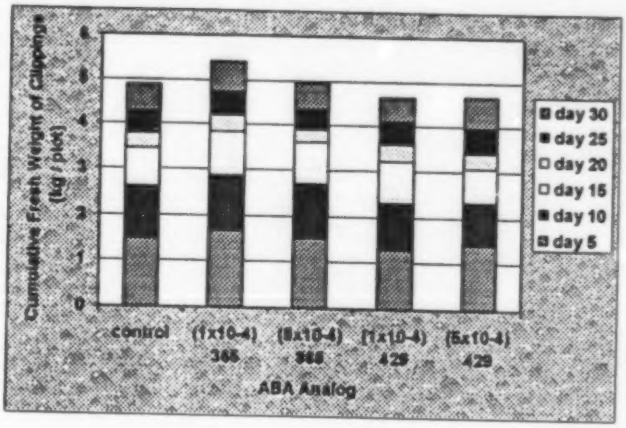


Figure 7.1: The effect of foliar-applied ABA analogs on cumulative fresh weight of turfgrass clippings after treatment

Leaf Blade Height of the Turfgrass

Treatment means for the influence of ABA analogs on turfgrass leaf blade height obtained from the test plots at varying intervals from the time of application are presented in **table7. 2**. For the first 20 days after application of the ABA analogs, the leaf blade heights of the control treatments were significantly lower than when ABA was applied. However, at 25 to 30 days after application of the ABA analogs, the leaf blade heights of the control were significantly higher than the ABA analog treatments. The high concentration of ABA treatments was more effective at suppressing leaf blade height at 25 days after the initial application than the lower ABA concentrations. Averaged over the entire trial, the ABA treatments were significantly different in relation to each other when comparing the turfgrass height. The higher ABA concentration treatments also showed an

Table 7.1: The effect of foliar-applied ABA analogs on fresh weight of turfgrass clippings at various intervals after treatment

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	5	10	15	20	25	30	
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ABA-429 (1x10^{-4} M)	1.29	1.01	0.91	0.37	0.53	0.53	0.77
ABA-429 (5x10^{-4} M)	1.40	0.93	0.73	0.35	0.54	0.71	0.78
P – value	0.00	0.00	0.00	0.01	0.01	0.14	0.15
LSD	0.20	0.18	0.10	0.07	0.10	0.12	0.21
	CONTRAST						
ABA analogs vs. control	0.94	0.61	0.56	0.36	0.74	0.64	0.94
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Concentration: [high] vs. [low]	0.84	0.35	0.00	0.05	0.29	0.35	0.60

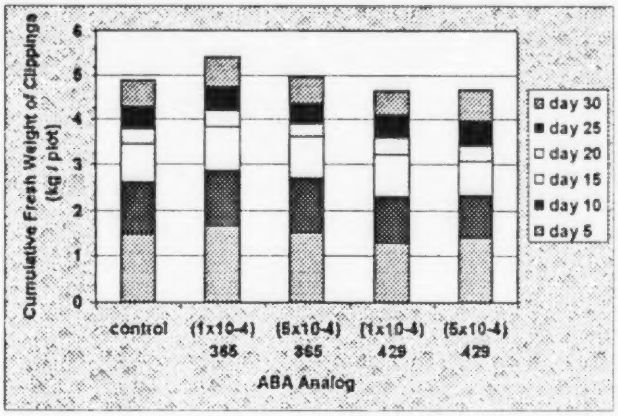

Figure 7.1: The effect of foliar-applied ABA analogs on cumulative fresh weight of turfgrass clippings after treatment

Leaf Blade Height of the Turfgrass

Treatment means for the influence of ABA analogs on turfgrass leaf blade height obtained from the test plots at varying intervals from the time of application are presented in **table7. 2**. For the first 20 days after application of the ABA analogs, the leaf blade heights of the control treatments were significantly lower than when ABA was applied. However, at 25 to 30 days after application of the ABA analogs, the leaf blade heights of the control were significantly higher than the ABA analog treatments. The high concentration of ABA treatments was more effective at suppressing leaf blade height at 25 days after the initial application than the lower ABA concentrations. Averaged over the entire trial, the ABA treatments were significantly different in relation to each other when comparing the turfgrass height. The higher ABA concentration treatments also showed an

overall average that significantly suppressed the turfgrass height more effective in relation to the lower ABA concentration treatments. The leaf blade heights of the turfgrass are presented in a cumulative graph in **figure 7.2**.

Table 7.2: The effect of foliar-applied ABA analogs on height of turfgrass at various intervals after treatment

Treatment And Concentration	Days After Initial Application				Average Height (cm)
	15	20	25	30	
		Height (cm)			
Control	4.17	4.18	7.58	7.34	5.82
ABA-365 (1x10^{-4}M)	4.70	4.64	7.34	6.94	5.91
ABA-365 (5x10^{-4}M)	5.66	4.84	6.52	6.46	5.87
ABA-429 (1x10^{-4}M)	5.31	4.90	7.42	7.12	6.19
ABA-429 (5x10^{-4}M)	4.71	4.60	6.46	6.02	5.45
P – value	0.00	0.00	0.00	0.00	0.00
LSD	0.43	0.38	0.38	0.37	0.30
CONTRAST					
ABA analogs vs. control	0.00	0.00	0.00	0.00	0.77
ABA-365 vs. ABA-429	0.28	0.94	0.94	0.33	0.51
Concentration: [high] vs. [low]	0.25	0.71	0.00	0.00	0.00

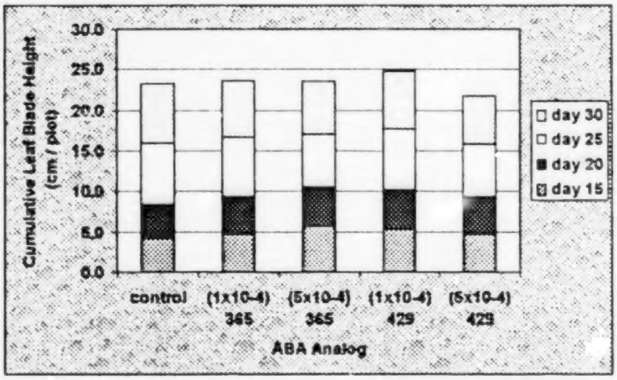

Figure 7.8: The effect of foliar-applied ABA analogs on the cumulative leaf blade height of turfgrass after treatment

Discussion

The experimental hypothesis tested in this trial was that the application of ABA analogs would maintain the visual quality while reducing plant growth of the turfgrass. The ABA-365 and ABA-429 analogs were studied at various concentrations to determine which treatment was the most effective as a turfgrass PGR. To be an effective PGR, the ABA analogs must consistently suppressed plant growth while maintaining the visual appearance of the turfgrass. Any reductions in turfgrass growth would indicate reduced maintenance cost such as mowing and irrigation. The visual quality of the turfgrass was not effected by any of the ABA analog treatments. During the entire test period, overall average that significantly suppressed the turfgrass height more effective in relation to the lower ABA concentration treatments. The leaf blade heights of the turfgrass are presented in a cumulative graph in **figure 7.2**.

Table 7.2: The effect of foliar-applied ABA analogs on height of turfgrass at various intervals after treatment

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ABA-365 (5x10^{-4}M)	5.66	4.84	6.52	6.46	5.87
ABA-429 (1x10^{-4}M)	5.31	4.90	7.42	7.12	6.19
ABA-429 (5x10^{-4}M)	4.71	4.60	6.46	6.02	5.45
P – value	0.00	0.00	0.00	0.00	0.00
LSD	0.43	0.38	0.38	0.37	0.30
CONTRAST					
ABA analogs vs. control	0.00	0.00	0.00	0.00	0.77
ABA-365 vs. ABA-429	0.28	0.94	0.94	0.33	0.51
Concentration: [high] vs. [low]	0.25	0.71	0.00	0.00	0.00

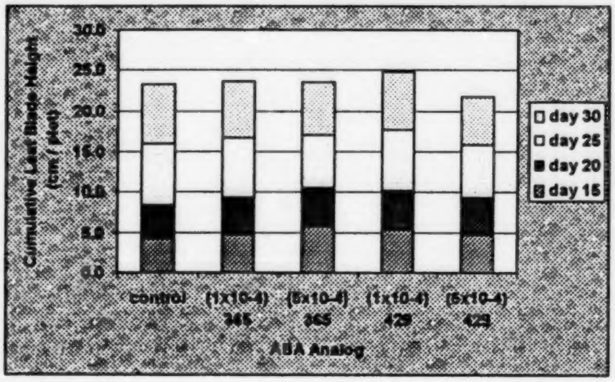

Figure 7.8: The effect of foliar-applied ABA analogs on the cumulative leaf blade height of turfgrass after treatment

Discussion

The experimental hypothesis tested in this trial was that the application of ABA analogs would maintain the visual quality while reducing plant growth of the turfgrass. The ABA-365 and ABA-429 analogs were studied at various concentrations to determine which treatment was the most effective as a turfgrass PGR. To be an effective PGR, the ABA analogs must consistently suppressed plant growth while maintaining the visual appearance of the turfgrass. Any reductions in turfgrass growth would indicate reduced maintenance cost such as mowing and irrigation. The visual quality of the turfgrass was not effected by any of the ABA analog treatments. During the entire test period, the turfgrass remained healthy looking, with green leaf blades. There was no increase in the incidence of weeds within the test plots, indicating that the ABA treatments had no effect on the competitive ability of the turfgrass.

For the first 20 days after application of the ABA analogs, the treatments appeared to stimulate growth as indicated by increasing plant height in relation to the control. However, at 25 to 30 days after the application of ABA, the treatments significantly suppressed the leaf blade height in relation to the control. Averaged over the duration of the trial, the ABA treatments had no effect on turfgrass height. There is no previous research indicating why ABA treatments might initially stimulate plant growth and then subsequently reduce growth. The fresh weight data did not show any corresponding effect of the ABA treatments. This suggests limited treatment effects on photosynthesis. Hopkins (1999), Monotague (1997), and Roberts et al. (1988) indicated that ABA reduced plant growth by the closure of stomata preventing water loss, gas exchange, and thereby reducing photosynthesis within the leaf blade.

The ABA-365 and ABA-429 analogs were compared to determine their relative effect on the turfgrass. Abrams et al. (1997) indicated that the ABA-365 analog is more active, persistent, and has a slower metabolism than the standard ABA. Churchill et al. (1998) indicated that the ABA-429 analog has shown to maintain or gain effectiveness over time. The ABA-365 analog at low concentrations (1×10^{-4}) was more active at suppressing turfgrass growth. The ABA-429 analog at high concentrations (5×10^{-4}) was more active at suppressing turfgrass growth. The degree of persistence fluctuated and was similar between the ABA-365 and ABA-429 analogs during the entire test period.

Two concentrations of the ABA were studied to determine if the level of applied ABA had any influence on turfgrass response. The high concentrations of the ABA analog treatments seemed to be more effective at suppressing turfgrass growth than lower concentrations. Hopkins (1999) and Roberts et al. (1988) indicated higher ABA levels in plant tissues was more effective at suppressing plant growth. The high ABA concentration showed no apparent phytotoxic effects with no signs of leaf blade discoloration or reduced quality of the turfgrass. Higher concentrations of ABA may be longer lasting and seem to be more effective at suppressing turfgrass growth. The limitation of higher concentrations of ABA applications is the extra cost of the chemicals.

Conclusion

The weather greatly influences the effect of ABA as a PGR treatment. Favorable growing conditions enhance the productivity of the turfgrass and counteract the effect of PGR's such as ABA. Applications of ABA during warm dry weather should be more effective at reducing the growth of the turfgrass, as a lack of soil moisture causes the stomata to close. In this trial, the ABA analog treatments produced no consistent reduction in the growth of turfgrass as indicated by clipping fresh weight and leaf blade height of the turfgrass. The fact that higher ABA concentrations were marginally more effective than lower concentrations suggests that the concentration tested may not have been adequate. Higher concentrations or a more biologically active ABA analog may produce more dramatic results. Even higher concentrations of the ABA treatments showed no adverse effects on the quality of the turfgrass. This again suggests the potential for using higher concentrations of ABA. The conclusion from this study is that further research is required for ABA analogs to be effective as PGR's for turfgrass.

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